# EVALUATION OF THE EFFECT OF PULSED ELECTRO MAGNETIC FIELD THERAPY IN TYPE-2 DIABETES MELLITUS PATIENTS WITH PAINFUL SENSORY POLYNEUROPATHY

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**The Tamil Nadu Dr. MGR Medical University**

***In partial fulfilment of the regulations for the award of the degree of***

## M.D. PHYSIOLOGY

## Branch V


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**Madras Medical College and Rajiv Gandhi Government General Hospital**

**CHENNAI –600003**

**THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY**

**CHENNAI –600032**

**APRIL 2016**

# CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF THE EFFECT OF PULSED ELECTRO MAGNETIC FIELD THERAPY IN TYPE-2 DIABETES MELLITUS PATIENTS WITH PAINFUL SENSORY POLYNEUROPATHY"** by the candidate

**Dr. CHARUMATHI VENKATESHWARLU** for M.D Physiology is a bonafide record of the research done by her during the period of study (2013 – 2016) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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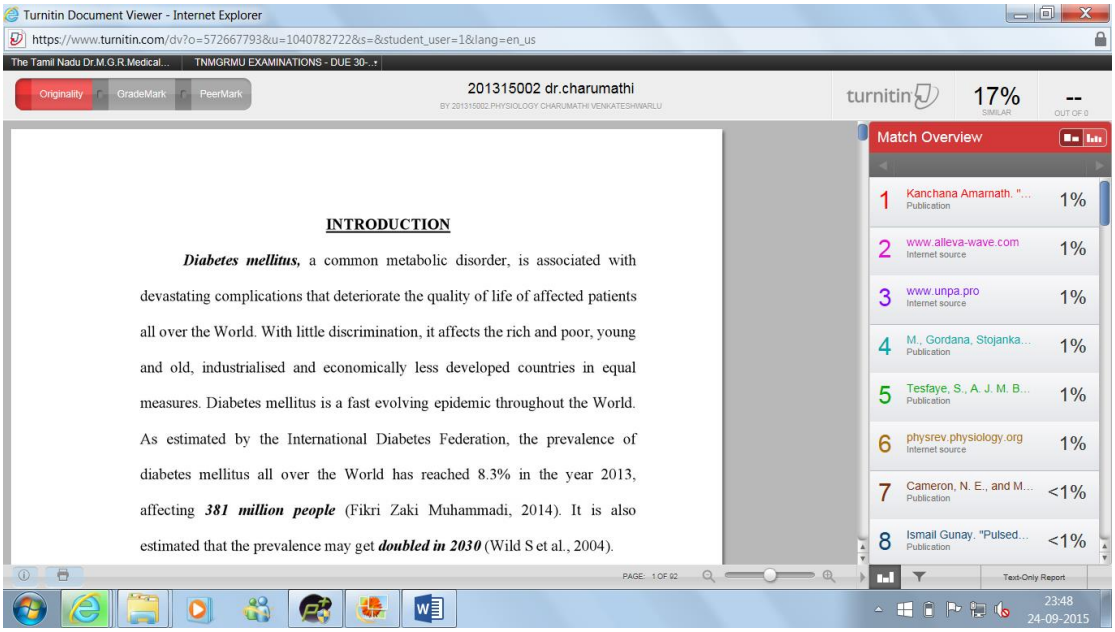
## ABBREVIATIONS

| | |
|---|---|
| AGE | Advanced Glycation End products |
| CaM | Calmodulin |
| DNSS | Dyck's Neuropathic Symptom Score |
| DPN | Diabetic Peripheral Neuropathy |
| DSPN | Distal Symmetrical Poly Neuropathy |
| FGF-2 | Fibroblast Growth Factor-2 |
| GSH | Glutathione |
| NCV | Nerve Conduction Velocity |
| NGF | Nerve Growth Factor |
| NOS | Nitric Oxide Synthase |
| NO | Nitric Oxide |
| PEMF | Pulsed Electro Magnetic Fields |
| PLA | Phospholipid autoantibodies |
| RNS | Reactive Nitrogen Species |
| ROS | Reactive Oxygen Species |
| SNAP | Sensory Nerve Action Potential |
| SOD | Super Oxide Dismutase |
| STZ | Streptozocin |
| Tc-99 MDP | Technicium-99 Methylene di phosphonate |
| VAS | Visual Analog Score |
| VPT | Vibration Perception Threshold |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INTRODUCTION
Diabetes mellitus, a common metabolic disorder, is associated with devastating complications that deteriorate the quality of life of affected patients all over the World. With little discrimination, it affects the rich and poor, young and old, industrialised and economically less developed countries in equal measures. Diabetes mellitus is a fast evolving epidemic throughout the World. As estimated by the International Diabetes Federation, the prevalence of diabetes mellitus all over the World has reached 8.3% in the year 2013, affecting 381 million people (Fikri Zaki Muhammadi, 2014). It is also estimated that the prevalence may get doubled in 2030 (Wild S et al., 2004).
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## ABSTRACT

**EVALUATION OF THE EFFECT OF PULSED ELECTRO MAGNETIC FIELD THERAPY IN TYPE-2 DIABETES MELLITUS PATIENTS WITH PAINFUL SENSORY POLYNEUROPATHY**

| | |
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| Supervisor and guide | : Prof. Dr. K. Padma<br>Director and Head of the Department |
| Department | : Institute of Physiology and Experimental Medicine |
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| University | : The Tamil Nadu Dr. M. G. R. Medical University, Chennai-600032 |
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***Background:***

Painful diabetic neuropathy, involving the lower limbs which is worse at night interferes with normal sleep and affects the quality of life. Pharmacotherapies used for diabetic neuropathy are not recommended for use in older adults and patients with heart disease. Pulsed Electro Magnetic Field (PEMF) therapy, has gained clinical interest in the present-day and is found to have analgesic, neuro stimulatory, neurotrophic actions.

It is also observed that they modulate pain, influence nerve regeneration by depolarising, hyperpolarising or repolarising neurons.

***Aim and objectives:***

To determine whether low frequency PEMF therapy can reduce neuropathic pain and influence nerve regeneration and improve vascularity of foot in type-2 diabetes mellitus patients with painful sensory polyneuropathy.

***Methods and Methodology:***

The study included thirty patients of type-2 diabetes mellitus for more than ten years duration with painful neuropathy. They were subjected to PEMF therapy, delivering 1500 nTesla (10Hz frequency with square wave configuration) for 60 minutes/ day for 21 days with a break after every 6 days. Before and after the PEMF therapy, pain assessment by Visual Analog Scale (VAS), assessment of Vibration Perception Threshold (VPT) of foot, nerve conduction study in superficial peroneal nerve, erythrocyte Super Oxide Dismutase (SOD) Levels and Tc-99 MDP three phase bone scan were performed.

***Results:***

After the Pulsed Electro Magnetic Field therapy, there was a significant reduction ($p<0.001$) in Visual Analog Score and latency of Sensory Nerve Action Potential (SNAP) in Superficial Peroneal Nerve and a significant improvement ($p<0.001$) in the Vibration Perception Threshold, Nerve Conduction Velocity (NCV)

and amplitude of SNAP in Superficial Peroneal Nerve. An increase in the peripheral blood flow indicated by significant increase in Integral 60-120 seconds, frequency of counts in blood pool, one hour delay, three hour delay and counts in bone scan along with reduction of Time to ½ Max. in Tc-99 MDP three phase bone scan were also observed. Erythrocyte Super Oxide Dismutase (SOD) levels has shown a significant increase ($P<0.001$) following PEMF therapy.

***Conclusion:***

This study shows that PEMF improves peripheral circulation of foot in painful diabetic polyneuropathy, thereby reducing pain and influencing nerve regeneration. It also reduces oxidative stress by increasing Super Oxide Dismutase (SOD) levels. Hence, PEMF which addresses the pathogenesis of diabetic neuropathy rather than merely focusing on pain alleviation could be used as a potential treatment modality for treating painful diabetic neuropathy. It has the advantage of non-invasiveness, involves no medications and adverse effects.

***Key words:*** Pulsed Electro Magnetic Field (PEMF) therapy, Painful Diabetic Neuropathy, Nerve Conduction Study, Superficial Peroneal Nerve, Vibration Perception Threshold, Visual Analog Scale, erythrocyte Super Oxide Dismutase levels.

# INTRODUCTION

***Diabetes mellitus,*** a common metabolic disorder, is associated with devastating complications that deteriorate the quality of life of affected patients all over the World. With little discrimination, it affects the rich and poor, young and old, industrialised and economically less developed countries in equal measures. Diabetes mellitus is a fast evolving epidemic throughout the World. As estimated by the International Diabetes Federation, the prevalence of diabetes mellitus all over the World has reached 8.3% in the year 2013, affecting ***381 million people*** (Fikri Zaki Muhammadi, 2014[1]). It is also estimated that the prevalence may get ***doubled in 2030*** (Wild S et al., 2004[2]).

The aetiology of Diabetes Mellitus is hyperglycaemia produced by insulin deficiency or insulin resistance. It generally presents with hyperglycaemia, polyuria, polydipsia and polyphagia. In long-term, this disease may damage nerves, kidneys, eyes, heart and blood vessels.

**Diabetes mellitus and diabetic foot problems in India:**

In India, more than ***62 million people*** are suffering from this recently growing epidemic, corresponding to 7.1% of its total Adult population (IANS[3]). Diabetes mellitus contributes to approximately ***1 million deaths*** in India every year (Vinik et al., 2000[4]). Diabetes mellitus in long term, may lead to microvascular and macro vascular diseases which are increasing in prevalence along with the rising epidemic of diabetes mellitus.

A prevalence study by ***Ramachandran et al.***[5] has shown the prevalence of vascular complications in type 2 diabetes in Indian subjects as follows:

| **Complication** | **Prevalence in Indian subjects (%)** |
|---|---|
| ***Peripheral neuropathy*** | ***27.5*** |
| Retinopathy | 23.7 |
| Heart disease | 11.4 |
| Nephropathy | 5.5 |
| Peripheral Vascular disease | 4.0 |
| Stroke | 0.9 |

It is clear from the above table that diabetic neuropathy, affecting 27.5% of diabetic population contributes to major disease burden. Peripheral neuropathy and associated recurring foot infections are very common in India (Viswanathan V et al.2000[6]). It has been estimated that approximately ***90,200 million rupees every year*** is spent on diabetes foot care (Shobana et al., 2000[7]). In diabetes patients, diabetic neuropathy is a frequent cause of morbidity and death (Vinik et al., 2000[4]). Triad of causal factors for diabetic foot ulcers include neuropathy with/ without ischemia, deformity and trauma (Viswanathan V et al.2000[6]). The peripheral neuropathy is the most important factor required to complete causal pathway to foot ulceration (Viswanathan V et al.2000[6], Singh N et al., 2005[8], Boulton AJ et al. 2004[9]). With the upsurge in the prevalence of diabetes in epidemic proportion, incidence of diabetic ulcer, the most frequent

complication affecting lower limbs predominantly has also increased. The lifetime risk of development of a foot ulcer can go up to 25% in patients with diabetes. Around 30 per cent of diabetic persons more than 40 years of age suffer from diabetic foot ulcers (Singh N et al., 2005[8]).

**Diabetic neuropathy:**

Diabetes is the most important cause of peripheral neuropathy, a disorder that affects the functioning of neurons (Boulton AJ et al. 2004[9]). Around ***50% of patients*** with diabetes suffer from neuropathy (Abbott CA et al., 2011[10]). Diabetic neuropathy most commonly presents as ***Distal Symmetrical Poly Neuropathy*** (DSPN) accounting for 75% of diabetic neuropathies (Boulton AJ et al. 2004[9]). Distal neuropathy is usually associated with progressive loss of both autonomic and somatic nerve fibres. Somatic fibres may be affected in a diffuse pattern leading to a ***sensory disorder*** or in a patchy fashion producing a ***motor disorder***.

**Painful diabetic neuropathy:**

Pain is a useful sensation that indicates an impending or actual tissue damage which in turn leads to aversive or attentive actions for protecting the body from harm. The after effects of losing the ability to perceive pain is seen in diabetic neuropathy. Painful diabetic neuropathy is the most frequent cause of neuropathic pain contributing to ***72% of neuropathy cases***, of which around 64% had moderate to severe pain and around 50% of the patients reported pain-related

interference in their daily activities (IndINeP Study Group, 2008[11]). Painful DSPN is ***nocturnal in nature*** and ***disturbs sleep*** (Zelman DC et al., 2006[12]). It adversely affects the normal physical and mental functioning of individuals and hence the quality of life. The risk of painful diabetic neuropathy is ***common in type 2 diabetes patients*** is 85% more than that of type 1 diabetes (Abbott CA et al., 2011[10]). Distressing tingling sensation, commonly known as dysesthesia is seen in almost half of the patients. Dysesthesia is usually described as ***"burning"*** or ***"shooting"*** or ***"electric-shock like"*** or ***"lancinating"*** or ***"knife-like"*** or ***"crawling"*** or ***"aching"*** sensations by the patients. Sometimes they may feel unusual sensations like feeling of ***"walking over pebbles or hot sand"***. Existing few epidemiological studies reveal that the prevalence of painful DSPN is around 10–26% (Abbott CA et al., 2011[10], Davies M et al., 2004[13], Davies M et al., 2006[14]). Nearly 12.5% of patients with diabetic neuropathy had never reported their symptoms to doctor and around 39% were not undergoing treatment for their pain (Daousi C et al., 2004[15]) indicating that there is a substantial ***under diagnosis and under treatment*** of painful neuropathic symptoms.

**Present treatment for painful diabetic neuropathy:**

At present only three drugs are approved by FDA for treating painful diabetic neuropathy, namely ***Pregabalin***, an anticonvulsant, ***Duloxetine***, Serotonin Norepinephrine Reuptake Inhibitor (SNRI), and ***Tapentadol***, an opioid/SNRI. These drugs are aimed at treating the pain rather than interfering with the pathogenic mechanisms responsible for painful diabetic neuropathy and

also known to cause disagreeable side effects. None of them are found to be more effective than the tricyclic antidepressants (Morello CM et al., 1999[16]) that were used in the past.

Hence, treating painful diabetic neuropathy poses a ***challenge for both the treating physicians and the patients*** and there is a search for a potential treatment that could aim at pathogenesis rather than just the pain alleviation (Bril V et al. 2011[17]).

**Pulsed Electro Magnetic Field (PEMF):**

Various research works focussing on the magnetic fields have proven their effect on various biological processes and its effectiveness in treating many clinical conditions (Shupak NM. 2003[18], Markov MS, 2007a[19], Markov MS, 2007b[20]). Application of PEMF of frequency ranging between ***0.1 Hz to 20 Hz*** in animals and humans have shown to produce electrophysiological, neurochemical and biochemical changes (P.V.Sanker Narayan et al., 1985[21]). Pulsed Electro Magnetic Field (PEMF) therapy, has gained clinical interest in the present-day and is found to have ***analgesic, neuro stimulatory, neurotrophic actions*** (Sisken BF et al., 1989[22], Sisken BF et al., 1993[23], Walker J et al., 1994[24], Macias MY et al., 2000[25], Mert T 2006a[26]). Literature has also advocated that PEMF has the ability of stimulating nerve growth, regeneration and recovering the function of nerves by in vitro studies and animal models (Walker JL et al., 1994[24], Macias MY et al., 2000[25], Mert T 2006b[27], Tasset I et al., 2012[28], Kim S et al., 2008[29]). It is also observed that they modulate pain and influence nerve

regeneration by ***depolarising, hyperpolarising or repolarising neurons.*** Animal studies which were aimed at repair of damaged peripheral nerves have shown PEMF exposure helps in hastening the nerve regeneration (B.F.Sisken et al., 1994[30]). Many studies have proved that pulsed electromagnetic fields (PEMF) are capable of modifying the nerve function of diabetic neuropathy patients (Wrobel MP et al. 2008[31], Szymborska-Kajanek A et al. 2010[32]). An ***element of pain reduction*** is also seen during magnetic field exposure which modulates both the exogenous and endogenous opioid systems (M.Kavaliers et al. 1991[33], E.Choleris et al. 2002[34], J.H. Jeong et al. 2000[35]). Nevertheless, the clinical application of PEMF is still under controversy (Bril V et al. 2011[17]). Hence, more research is required for confirming the therapeutic effects of PEMF on Diabetic Peripheral Neuropathy.

This study focusses on evaluating whether the low frequency and low intensity Pulsed Electro Magnetic Field (PEMF) therapy is effective in reducing neuropathic pain, is capable of influencing nerve regeneration and improving peripheral blood flow in type-2 diabetes mellitus patients with painful sensory polyneuropathy by assessing and comparing pain using Visual Analog Score (VAS), sensory nerve conduction in superficial peroneal nerve, Vibration Perception Threshold (VPT) of foot, erythrocyte Super Oxide Dismutase (SOD) levels, peripheral blood flow in foot using Tc-99 MDP 3 phase bone scan before and after administration of Pulsed Electro Magnetic Field Therapy.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## Diabetes mellitus:

Diabetes mellitus clinically presents as hyperglycaemia caused either by chronic and/or relative insufficiency of insulin (Mathis D et al. 2001[36]).

## Types of diabetes mellitus:

### Type 1 diabetes (Insulin-dependent diabetes mellitus):

Type 1 diabetes is characterized by hyperglycaemia produced due to a complex disease process that mostly occurs after sudden onset of an auto immune process owing to genetic and environmental factors (Davies JL et al. 1994[37]). The pancreatic β cells of the islets of Langerhans are destroyed during this process resulting in ***insufficient insulin*** production (Keenan HA et al. 2010[38]). Administration of exogenous insulin remains the main stay of treatment.

### Type 2 diabetes (Non-Insulin Dependent Diabetes Mellitus):

Most common cause of diabetes mellitus, contributing to 85% of total diabetes patients. Type 2 diabetes mellitus is characterised by ***peripheral insulin resistance*** associated with compensatory increase in insulin secretion by pancreatic islets ultimately resulting in reduced islet secretory function. Nevertheless, relative decrease in insulin secretion is the final event that is responsible for producing hyperglycaemia (Kahn SE et al., 1993[39]). Reduced peripheral insulin sensitivity is predominantly noticed in tissues like skeletal muscle, liver and adipose tissue because of metabolism and glucose uptake occurring in these tissues.

**Gestational diabetes:**

High blood glucose level in a pregnant women who is not previously diagnosed as diabetic. This type of diabetes poses great risk to both the mother and baby. Increased risk of developing type 2 diabetes in the later part of life may occur as a consequence of gestational diabetes.

**Complications of Diabetes Mellitus:**

<table>
<tr><td colspan="2">Acute metabolic complications</td></tr>
<tr><td colspan="2">• Diabetic ketoacidosis<br>• Non-ketotic hyperosmolar coma</td></tr>
<tr><td colspan="2">Long term vascular complications (Angiopathy)</td></tr>
<tr><td>Microvascular (Damage to small blood vessels)</td><td>Macro vascular (Damage to arteries)</td></tr>
<tr><td>• Eyes: Retinopathy<br>• Kidney: Nephropathy<br>• Nerves: Neuropathy</td><td>Heart: Affects Coronary blood vessels leading to myocardial infarction<br>Brain: Affects Cerebral blood vessels causing stroke</td></tr>
<tr><td colspan="2">Chronic complications of diabetes: Depression (Nouwen A et al. 2011[40], dementia (Cukierman T et al., 2005[41]) and sexual dysfunction (Adeniyi AF et al., 2011[42], Thorve VS et al., 2011[43])</td></tr>
</table>

**Diabetic Peripheral Neuropathy (DPN):**

Diabetic Peripheral Neuropathy, a heterogeneous disorder, affects the nervous system presenting with diverse clinical manifestations. An internationally accepted simple definition for DPN is “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the

exclusion of other causes". Confirmation of diagnosis of DPN can be done by using quantitative electrophysiology and sensory testing. Most common type of diabetic neuropathy is Distal Symmetrical Poly Neuropathy.

**Types of Diabetic Peripheral Neuropathy:**

A. Distal Symmetrical Poly Neuropathy (DSPN)

B. Radiculopathy

C. Mono neuropathy or mononeuritis multiplex

D. Autonomic neuropathy

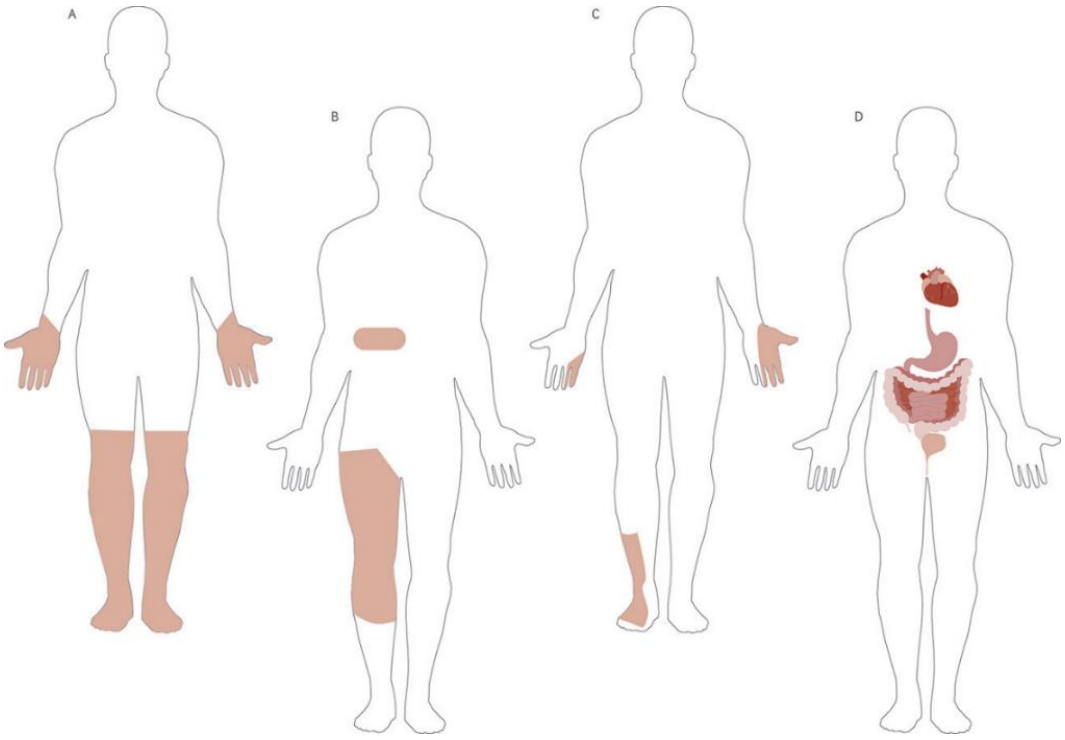



**Fig.1.Types of diabetic neuropathy.** A. Distal Symmetrical Poly Neuropathy (DSPN), B. Radiculopathy, C. Mono neuropathy or mononeuritis multiplex, D. Autonomic neuropathy

**Distal Symmetric Poly Neuropathy (DSPN):**

As defined by ***Tesfaye S et al.***[44], "DSPN is a symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and micro vessel alterations as a result of chronic hyperglycaemia exposure and cardiovascular risk covariates"

**Clinical features of DSPN:**

DSPN is usually gradual or insidious in onset which is indicated by ***sensory symptoms***. It involves the toes first followed by the feet and legs, spreading proximally. In severe cases, upper limb may also be involved in a similar fashion, beginning from the fingers. A proportion of patients also present with ***hyperalgesia, paresthesia and allodynia*** and around 40–50% of patients are found to suffer from pain due to diabetic neuropathy. Pain without any neuropathy on clinical examination is also seen in 10–20% of diabetes patients (Obrosova IG, 2009[45]). With the progression of the disease, motor weakness of lower limb muscles may also occur.

Some patients may even present with a foot ulcer, because the sensory loss is missed by the patient. Characteristic features of DSPN comprises of numbness, impaired pressure, vibration and joint position sensations. Presence of symptoms or signs of neuropathy along with nerve conduction abnormality is required for diagnosis of DSPN.

**Diabetic neuropathy Pathogenesis:**

Hypotheses concerning the aetiology of diabetic neuropathy have elaborated the following factors:

a. Metabolic insult to nerve fibres (Direct effect) (Greene DA et al. 1988[46])
b. Neurovascular insufficiency (Indirect effect) (Low PA, 1987[47], Cameron NE et al. 1994[48])
c. Impaired neurotrophic assistance (Vinik AI et al., 1995[49])
d. Damage produced by auto immune factors (Rabinowe SL et al. 1990[50])

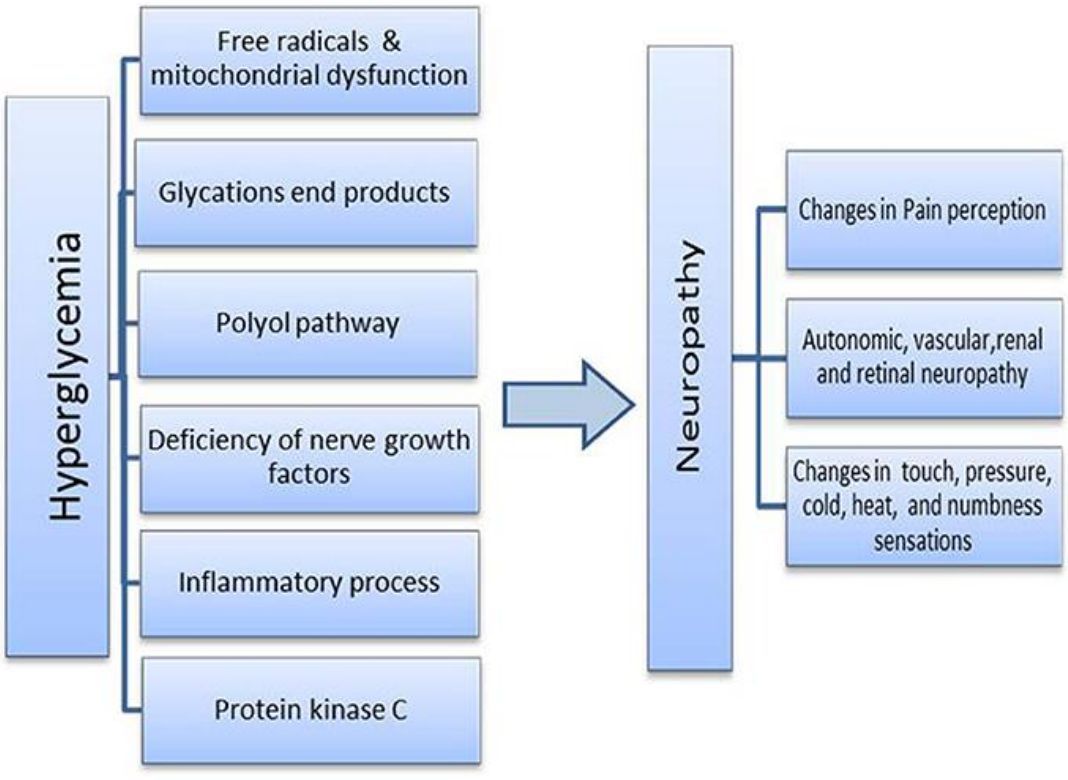



**Fig.2. Factors contributing to pathogenesis of diabetic neuropathy**

**Metabolic insult to nerve fibres:**

Majority of consequences of diabetes are mainly attributed to ***hyperglycaemia.*** Metabolic alterations in turn may lead to decrease in nerve blood flow and Nerve Conduction Velocity (Greene DA et al. 1988[46]). Various metabolic alterations in diabetic neuropathy (Cameron NE et al. 1994[48]) are:

a. Increased polyol pathway flux
b. Advanced glycosylation
c. Elevated oxygen free radical formation
d. Impaired metabolism of essential fatty acid (ω-6) and carnitine

**Polyol pathway:**

In normal individuals, reduction of glucose to sorbitol shields the cells against oxidative stress and derangement of NO action (Cameron NE et al. 1994[48]). Hyperglycaemia in diabetes leads to ***elevation in sorbitol levels*** which in turn gets converted into fructose, simultaneously converting NAD to NADH. Elevated sorbitol and fructose may lead to ***impaired nitric oxide,*** a vasodilator, leading to ischemia of nerve (Ando H et al., 2006[51]). Fructose is ten times better substrate for glycosylation in comparison to glucose. Advanced glycosylation end products (AGEs) formation would be hastened by increased polyol pathway flux (Brownlee M, 1992[52]). Furthermore, reduction in myo-inositol/ phosphoinositide metabolism has also been implicated (Greene DA et al. 1988[46]). Elevated sorbitol may result in an osmoregulatory compensation, leading to reduction in myo-inositol which may be explained by the compatible

osmolyte hypothesis ((Brownlee M, 1992[52]). Further, this may result in impaired phosphoinositide turnover and reduced stimulation of protein kinase C by di-acyl glycerol. Decreased stimulation of Protein Kinase C is in turn attributed to decrease in Nerve Conduction Velocity via reduced Na+-K+-ATPase activity (Greene DA et al. 1988[46]).

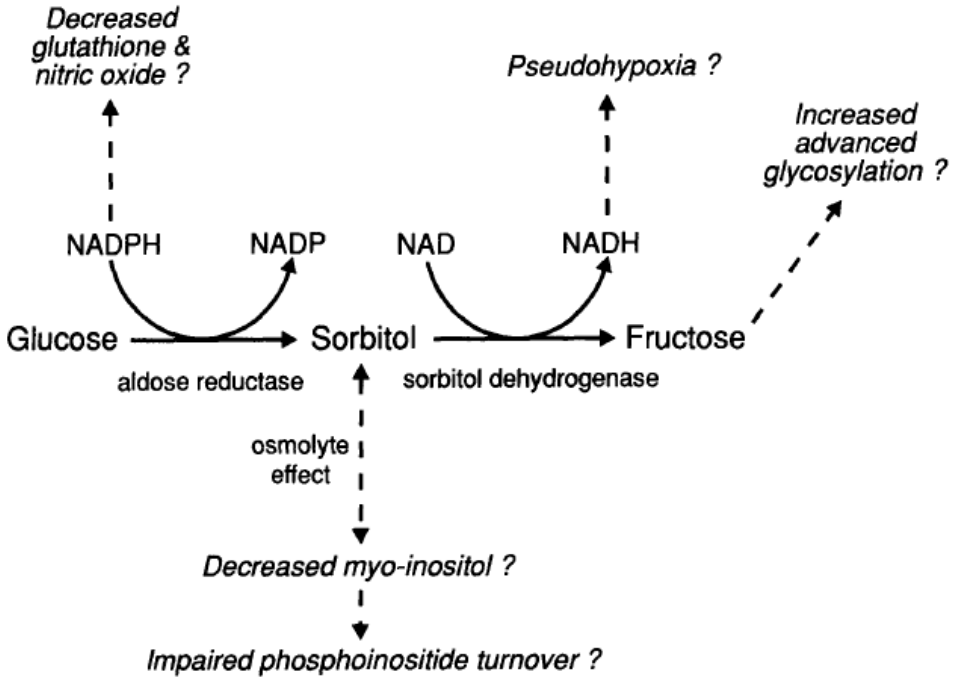



**Fig.3. Schematic diagram of polyol pathway in diabetic neuropathy**

**Effects of Advanced Glycosylation:**

The process of glycation takes place in two phases: early reversible phase producing Amadori products and late irreversible phase producing Advanced Glycation End products (AGEs).

**Glycosylation:**

Glycosylation is a type of enzymatic post translational modification. It is a site-specific and targeted process which involves the addition of glycans on to lipids, proteins and other organic molecules.

**N- Linked Glycosylation:**

N-linked glycosylation occurs within the endoplasmic reticulum (ER). Any defect in N-glycosylation may lead to accumulation of misfolded proteins intracellularly and improper trafficking of various intra cellular proteins. This phenomenon is called ER stress and is implicated as a pathological feature of diabetic complications (Kozutsumi Y et al., 1988[53]). The unfolded protein response activate three major signalling cascades, namely, protein kinase RNA (PKR)-like ER kinase (PERK), inositol requiring protein-1 (IRE1), and transcription factor-6 (ATF6) pathway.

ER stress protective pathways get overwhelmed in diabetes leading to initiation of pro apoptotic pathways (Kim R et al., 2006[54], Xu C et al., 2005[55]).

**O-Linked Glycosylation:**

O-glycosylation, a late posttranslational process that occurs within the Golgi apparatus (Haltiwanger RS et al.1998[56]), is a process involving addition (enzymatic) of galactosamine to serine or threonine residues followed by galactose or sialic acid. This is important for extracellular matrix proteins like

collagens and proteoglycans. In diabetic complications, there is excessive O-GlcNAc modification of proteins (Brownlee M, 2001[57]).

**Advanced Glycation:**

Advanced glycation is a non-enzymatic post translational modification of amino acids and free amino groups on proteins by covalent attachment of sugar moieties. This reaction, termed the "Maillard reaction" (Maillard L, 1912[58]), was first discovered hundred years ago. Some studies have shown that advanced glycation leads to modulation of insulin secretion (Coughlan MT et al., 2011[59] and signalling (Cassese A et al., 2008[60], Reddy MA et al., 2006[61]).

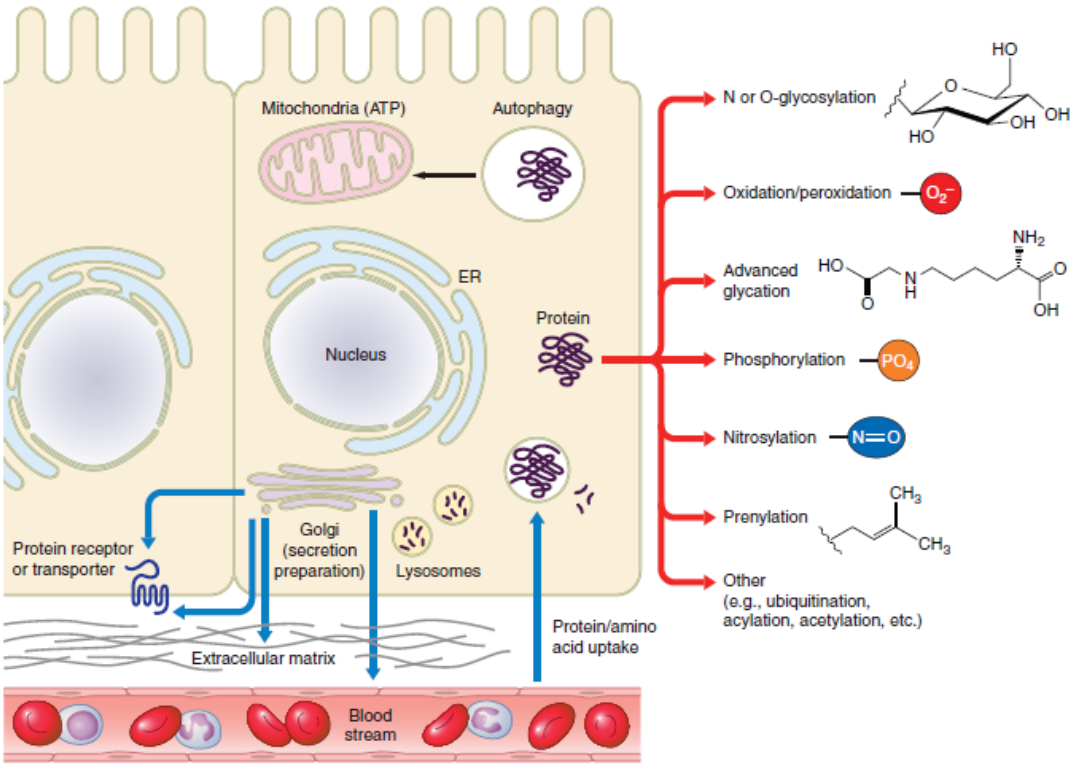



**Fig.4. Common post translational modifications in diabetes**

The intracellular AGE damages the target cells through 3 pathways:

1. **N-Glycosylation:** Modification of ***intracellular proteins*** and alteration of their function
2. **O-Glycosylation:** Modification of ***extra cellular matrix components*** leading to abnormal interaction with other matrix constituents and cell protein receptors
3. **Advanced glycosylation:** Modification of ***plasma proteins*** which bind to the AGE receptors present on various cells like endothelial, mesangial cells and macrophages leading to reactive oxygen species (ROS) generation (Fu MX et al., 1994[62]).

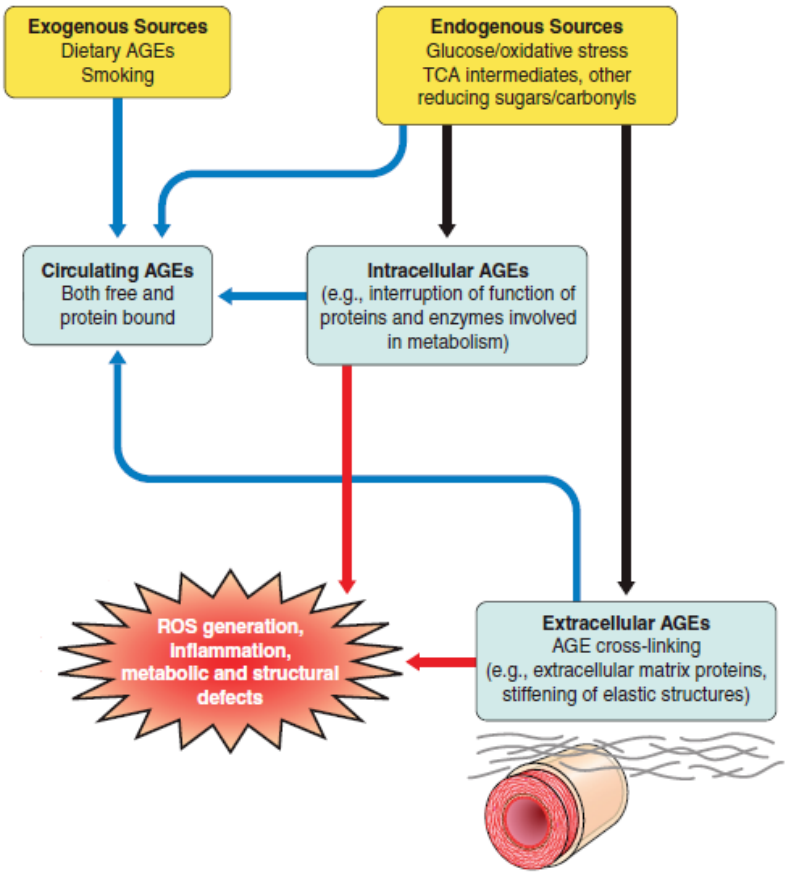



**Fig.5. Effect of Advanced Glycation End products**

**Free radicals:**

Atoms or molecules having one or more unpaired electrons are called free radicals. The presence of unpaired electron is responsible for their ***highly reactive and unstable nature*** (Halliwell, B, 1999[63]). Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are chief free radicals that damage cells. Free radicals are continuously produced in aerobic cells, most commonly as ROS. Free radicals produced are then eliminated by antioxidants defence constituting enzymes like catalase, superoxide dismutase and glutathione peroxidise.

| **Reactive Oxygen Species (ROS)[64, 65, 66, 67]** | **Reactive Nitrogen Species (RNS)** |
|---|---|
| Hydroxyl (OH) | Nitric oxide (NO) |
| Superoxide ($O^{2-}$) | Nitrogen dioxide ($NO_2^-$) |
| Hydro peroxyl ($HRO^{2-}$) | Peroxynitrite ($ONOO^-$) |
| Peroxyl ($RO_2$) | Nitrous oxide ($HNO_2$) |
| Hydrochlorous acid (HOCl) | Alkyl peroxynitrates (RONOO) |
| Hydrogen peroxide ($H_2O_2$) | |

**Anti-oxidant mechanisms:**

Super Oxide Dismutase (SOD) is an enzyme that catalyses the conversion of superoxide anion into hydrogen peroxide and oxygen.

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Activity of SOD was first discovered by McCord and Fridovich in 1969 (Szaleczky, E et al., 1999[68]). Consequently, they recognised that SOD was crucial in maintaining life in aerobic conditions (McCord et al., 1971[69]). The hydrogen peroxide is converted in to water by the enzyme glutathione peroxidase (GPx).

Microvascular disease in diabetes is found to alter the expression and functioning of antioxidant enzymes (Ceriello A et al., 2000[70], Hinerfeld D et al. 2004 [71], DeRubertis FR et al., 2007[72], Min D et al. 2012[73]).

**Super Oxide Dismutase:**

SOD is a metalloprotein made up of a metal ion, either Copper-Zinc (Cu-Zn), Manganese (Mn), Iron (Fe) or Nickel (Ni) (Fridovich, I, 1998[74]) at its centre.

Fe-SOD is usually seen in prokaryotes and plants. In humans, intra and extra cellular forms of Cu-Zn SOD and mitochondrial Mn SOD have been recognised.

Intracellular ***Cu-Zn SOD*** is made up of two identical subunits (Tainer et al., 1982[75]), each comprising of a Cu (II) and a Zn (II) ion. Catalytic activity of SOD is due to Cu (II) while stabilization of enzyme conformation is done by Zn (II) (Djordjević, B. V et al., 2000[76]). Hydrogen peroxide inactivates cytosolic Cu-Zn SOD by converting Cu (II) into Cu (II)–OH or the ionized form Cu (II)–$O^-$.

Extracellular SOD ***(ECSOD)*** is a tetrameric glycoprotein. Each subunit consists of a Cu and Zn atom. ECSOD exhibits high affinity for heparin sulphate, facilitating high concentration of ECSOD in specific regions like extracellular

space and on the cell surface. Expression of ECSOD is primarily increased by IFN γ and reduced by TNF α and TGF β. Decreased ECSOD expression in turn results in reduced mitochondrial GSH and increased oxidative stress (Lebovitz, R.M et al. 1996[77]).

Mitochondrial SOD ***(MnSOD)*** is found in the mitochondrial matrix. Two isoforms of MnSOD, dimeric MnSOD and tetrameric MnSOD have been identified. Each subunit of MnSOD consists of one Mn (III) ion. It is usually produced in a constitutive manner. Production of Mn (III) can be stimulated by IL-1, TNF or an endotoxin (Tang, L et al., 1994[78]).

**Oxidative stress:**

Imbalance between production of reactive oxygen species (ROS) and antioxidant capacity may lead to oxidative stress. Oxidative stress can result either from increase in generation of ROS or any defect in functioning of antioxidant system.

**Effect of oxidative stress in diabetic neuropathy:**

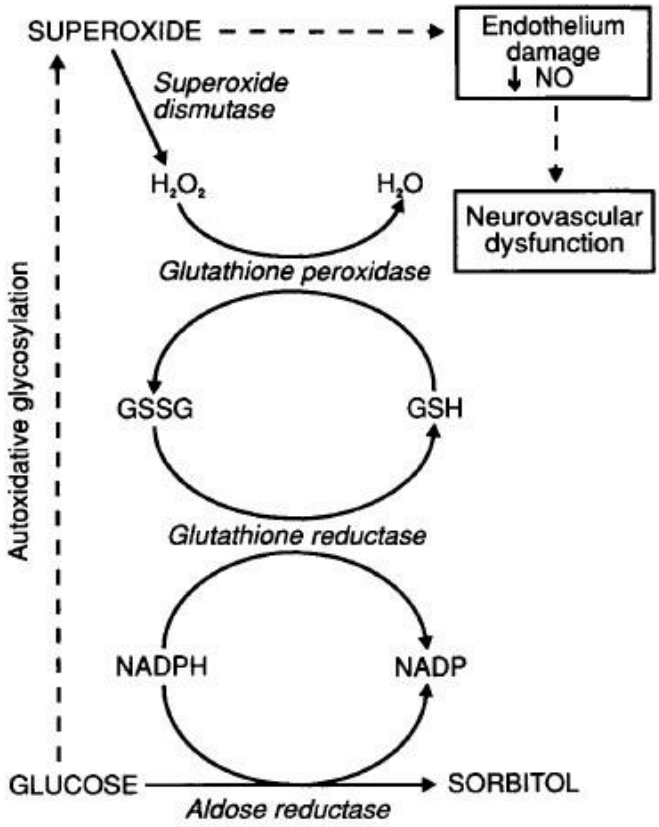
Chronic hyperglycaemia is responsible for oxidative stress by enzymatic, non-enzymatic and mitochondrial pathways.

**Non-enzymatic pathway:**

Glucose reacts with proteins through a non-enzymatic process to reduce molecular oxygen to highly reactive superoxide radical, hydrogen peroxide, and hydroxyl radical.

**Enzymatic pathway:**

The main source of reactive oxygen species in diabetes is autoxidation of excess glucose which is catalysed by free transition metal ions including iron or copper present in trace amounts. Another source of free radicals is AGEs (Fu MX et al., 1994[62]). When endothelial cells were cultured in high glucose environment, they exhibited an abnormal GSH redox cycle, and reduction in NADPH and GSH levels and viability of endothelial cells were reduced when they were given a peroxide challenge (Kashiwagi A et al., 1994[79]).




**Schematic of a putative mechanism whereby increased flux through the first half of the polyol pathway can reduce the effectiveness of the glutathione redox cycle in scavenging oxygen free radicals, leading to neurovascular dysfunction. GSSG, oxidized form of glutathione; GSH, reduced form of glutathione; NADP(H), nicotinamide-adenine dinucleotide phosphate.**

**Fig. 6. Role of oxidative stress in causing neurovascular dysfunction**

Cofactor for aldose reductase, NADPH, is reduced due to elevated polyol pathway flux. This would in turn would impair the GSH redox cycle, resulting in significant increase in oxygen free radicals. This is followed by damage to the vascular endothelium and neutralization of Nitric Oxide, leading to reduction in vasa nervorum vasodilation. Superoxide radicals are removed enzymatically by Super oxide dismutase. During excessive production of superoxide radicals, as in diabetes, they react with nitric oxide to form peroxynitrite that causes neuronal damage (Djordjević, B. V et al., 2000[76]).

**Mitochondrial pathway:**

Various studies have proven the mitochondrial functional abnormalities at regions of diabetic complications (Bugger H et al., 2009[80], Chowdhury SK et al., 2012[81], Coughlan MT et al., 2009[82], Roy Chowdhury SK et al., 2012[83], Shenouda SM et al. 2011[84], Tewari S et al. 2012[85], Zhong Q et al., 2012[86]). In humans, more than 90% of oxygen metabolized during oxidative phosphorylation, a process in which glucose and other metabolites donate electrons for reduction of molecular oxygen resulting in ATP production, occurs in mitochondria. This is a highly regulated process. Under physiological conditions, only less than 1% of oxygen is partially reduced to form $O_2^-$. Remaining is converted to water by antioxidant enzymes. The major sites of electron leakage that produces superoxide within the mitochondria are NADH dehydrogenase (complex I) and interface between coenzyme Q (CoQ) and complex III (Turrens JF et al. 1980[87]). In diabetes, superoxide ($O_2^-$) production

by dysfunctional mitochondria is being suggested as the principal initiating event in the process of development of diabetic complications (Nishikawa T et al., 2000[88]).

**Effect of essential fatty acids & L-carnitine derivatives:**

Hepatic A-6 desaturation of linoleic acid, main dietary source of ω6 essential fatty acids, is reduced in diabetes. Subsequently, this results in reduced synthesis of vasoactive prostanoids like $PGI_2$ by vasa nervosum of diabetic rats (Ward KK et al., 1989[89]).

**Neurovascular insufficiency in diabetic neuropathy:**

Reduced sciatic nerve blood flow have been observed in experimentally induced diabetes by many researchers using a variety of tests like laser-Doppler flowmetry, [$C^{14}$] butanol and [$C^{14}$] iodoantipyrine accumulation test and hydrogen clearance microelectrode polarography (Cameron NE et al., 1994[48]). Recent researches have established the presence of endoneurial hypoxia, resulting in reduced nerve conduction velocity (NCV) (Tuck RR et al., 1984[90], Maxfield EK et al., 1993[91], Cameron NE et al., 1994[92], Cameron NE et al., 1994[93]) and impaired sural nerve blood flow in neuropathy patients (Tesfaye S et al., 1994[94]).

Various functional defects found in the dermal microvasculature of diabetic subjects are ***decreased tissue $pO_2$, reduced microvascular blood flow***, ***increase in vascular resistance*** and ***altered characteristics of vascular***

***permeability***. These result from impaired vasodilator responses to Substance P and calcitonin gene–related peptide (CGRP) and reaction to nociceptive stimulation.

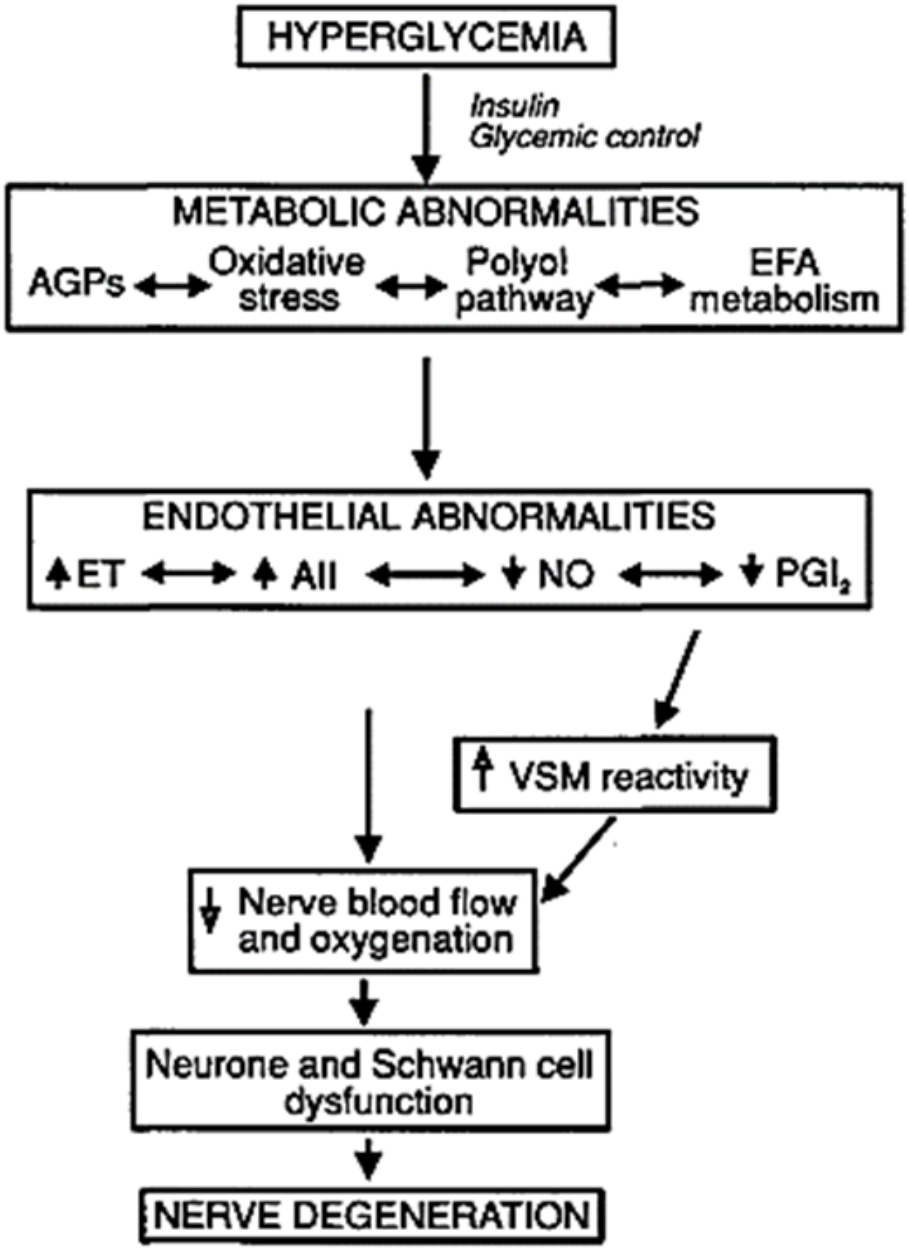



**Fig.7. Causal relationship between metabolic changes and peripheral nerve blood flow in diabetic neuropathy.** AII- Angiotensin II, EFA- Essential Fatty Acid, ET- Endothelin-1, $PGI_2$- Prostacyclin, VSM- Vascular Smooth Muscle

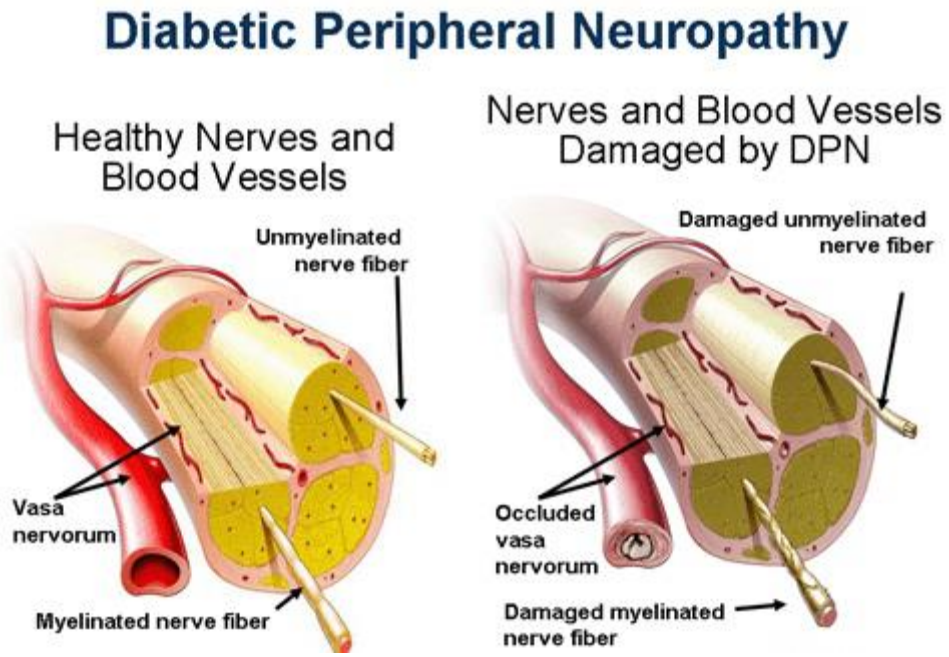



**Fig.8.** Neurovascular insufficiency in Diabetic neuropathy

**Impaired neurotrophic assistance:**

The neurons depend on the growth factors that are released from target tissue throughout life for their functioning, maintenance and survival. Recent studies have elucidated that sympathetic neurons and Dorsal Root Ganglion neurons are dependent on ***Nerve Growth Factor*** for maintenance (Calcutt, N. A. et al. 1990 [95] and survival (Rich, K. M. et al., 1987[96]). These group of neurons are most commonly affected in diabetic neuropathy. Nerve Growth Factor also appears to be an important regulator of adult DRG neurons synthesising Substance P (Lindsay,R. M. et al., 1989[97], Schwartz, J. P. et al., 1982[98]) which plays a major role in vasodilatation, gut motility, and nociception, affected in diabetic neuropathy. A dramatic reduction in Nerve Growth Factor levels are found in superior cervical ganglion, a NGF-dependent group of neurons in

Streptozotocin (STZ)-induced diabetic rats (Steinbacher-BC et al., 1998[99]), that usually represents a syndrome mimicking type 2 diabetes. A study by ***Hellweg et al.,***[100] has shown a reduction in retrograde transport of Nerve Growth Factor in the sciatic nerve in STZ-induced diabetic rats.

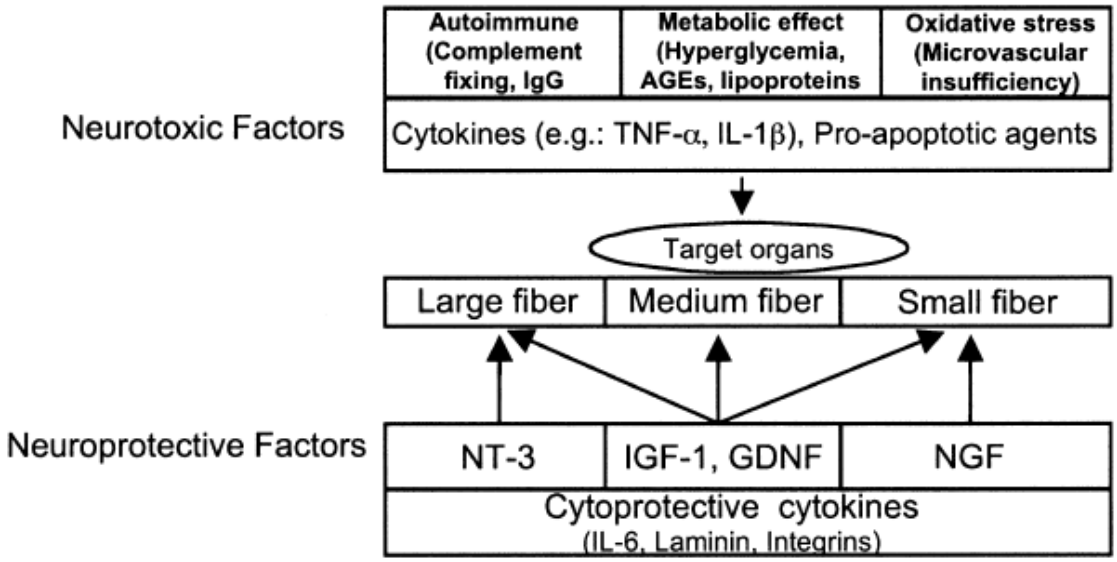



**Fig.9.** Balance between nerve destruction and neuroprotection in diabetic neuropathy

**Damage produced by auto immune factors:**

Phospholipid autoantibodies (PLAs) constitute a family of closely related IgG, IgM, and IgA immunoglobulins that interact with negatively charged phospholipids (Triplett DA et al. 1988[101], McNeil HP et al., 1991[102]). These PLAs are found to cross-react with neural phospholipids like sphingomyelin and cephalin, thereby damaging neural structures (Harris EN et al., 1985[103], Harris EN et al., 1988[104]). ***Phospholipid Autoantibodies (PLA)*** are found in 88% of diabetic neuropathy patients (Aaron i. Vinik, md, phd et al., 1995[105]).

**Painful Diabetic Neuropathy:**

***Rollo (1798):*** First person to describe the diabetic neuropathic pain as "pain and paraesthesia found in the legs of a diabetic patient" (Boulton AJ et al., 2004[9])

***Pavy (1887):*** Described diabetic neuropathy pain as a "pain of a burning and unremitting nature"

International Association for the Study of Pain has given a definition for peripheral neuropathic pain due to diabetes as "the pain arising as a direct consequence of abnormalities in the peripheral somatosensory system in people with diabetes." (Treede RD et al., 2008[106])

**Types of pain in diabetic neuropathy:**

Asbury and Fields have proposed a hypothesis that identifies specific painful symptoms of chronic painful diabetic peripheral neuropathy (Abbott CA et al., 2011[10]).

3 types of pain have been described by the hypothesis.

- Superficial pain
- Deep pain
- Muscle pain

**Superficial type of pain:**

Sensations like burning, allodynia and tingling arising from cutaneous or subcutaneous receptors are ascribed to nociceptive fibres that are damaged, sprouting and regenerating fibres that fire at increased rates.

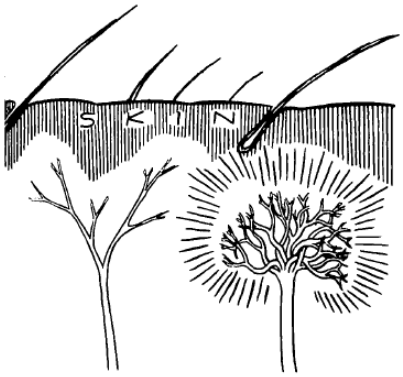
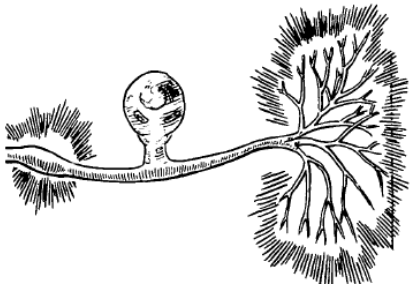
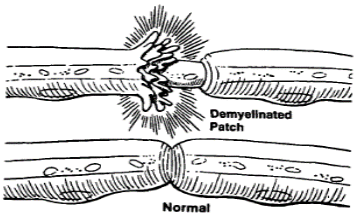
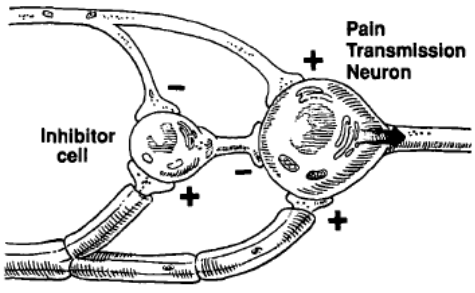
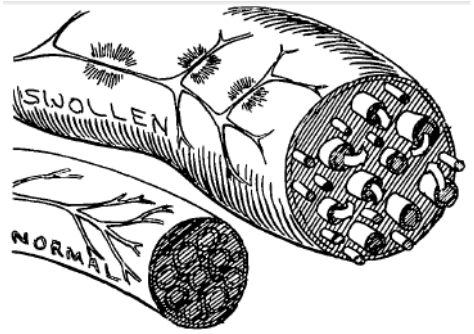



**Fig.10. Mechanism of superficial pain.** Increased firing of damaged or abnormally excited nociceptive fibres, particularly sprouting regenerating fibres

**Deep pain:**

This pain arises from deeper anatomical structures. It is described as "pins and needles" or "electric-like" (Fields HL, 1987[107]). This pain results from:

| | |
|---|---|
| **Spontaneous activity** and increase in mechanosensitivity of damaged afferents in dorsal root ganglion |  |
| **Ectopic impulses** produced by demyelinated patches of myelinated axons |  |
| **Modified gate control hypothesis:** Loss of segmental inhibition of myelinated fibres (large) and unmyelinated pain fibres (small) |  |

| | |
|---|---|
| **Increased firing of neurons** due to physiological stimulation of nociceptive afferents that innervate the nerve sheaths (nervi nervorum) |  |

**Muscular pain:**

Described as a “cramping pain”, “aching pain”, “muscle tenderness” or a "drawing sensation". This may be due to injury to motor nerves or due to a reflex loop "**Livingston's vicious circle**" (Livingston WK, 1943[108]). In this, an input, mostly nociceptive, activates the motor neuron in the spinal cord producing muscle spasm which in turn activates the nociceptors present in the muscle fibres and send feedback signal to the spinal cord and sustain the spasm.

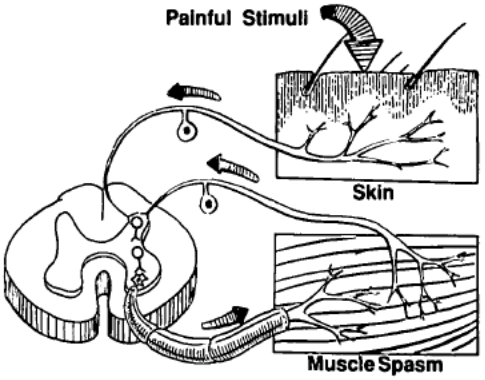



**Fig.11. Livingston’s vicious circle.** Involves a nociceptive input that activates the motor neuron within the spinal cord causing muscle spasms, in turn activating the muscle nociceptors and feeds back to the spinal cord to sustain the muscle spasm

**Mechanism of neuropathic pain in diabetes:**

Despite several peripheral nerve studies, the genesis of diabetic neuropathic pain remains poorly understood. The precise pathophysiological mechanism that is responsible for neuropathic pain in diabetes could not be defined, although several mechanisms have been suggested (Tesfaye S et al., 2005[109])

***Proposed mechanisms of pain generation in Painful diabetic neuropathy:***

- Inappropriate or exaggerated peripheral sensory neurons activity
- Alteration in sensory processing within the spinal cord
- Spontaneous activity in the central nervous system which is perceived as pain arising from the periphery

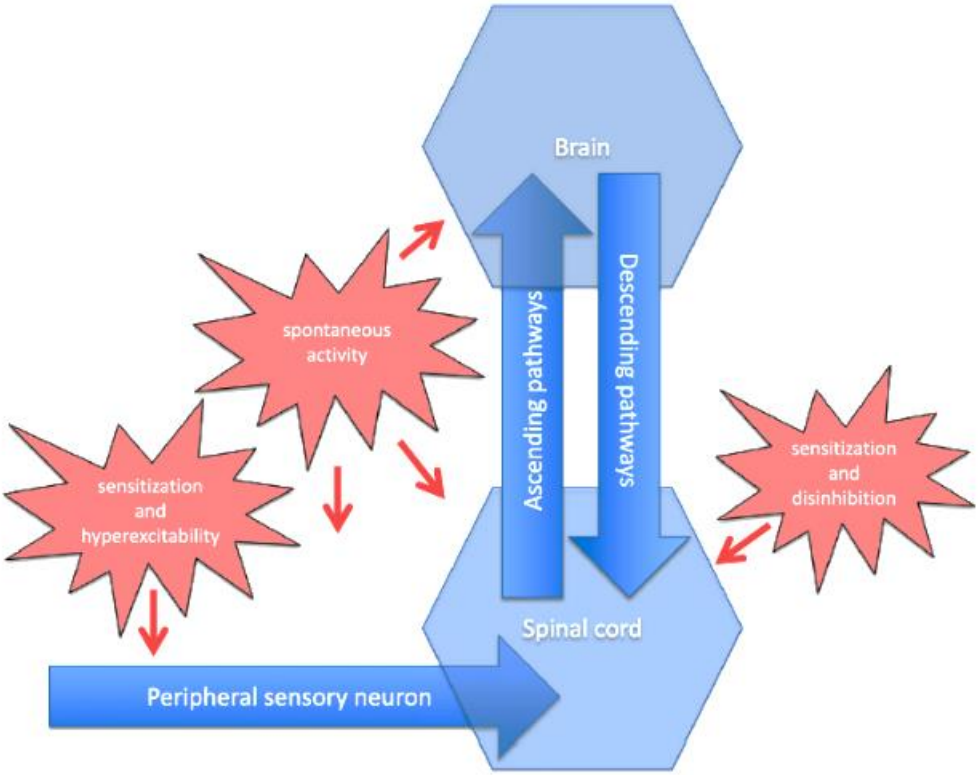



**Fig.12.** Proposed mechanisms of pain generation in Painful diabetic neuropathy

The following mechanisms of genesis of pain in diabetic neuropathy was proposed by Tesfaye and Kempler (Tesfaye S et al., 2005[109]).

**Peripheral mechanisms**

- Changes in sodium channel distribution and expression
- Changes in calcium channel distribution and expression

**Central mechanisms**

- Central sensitization
- Changes in the balance of facilitation/ inhibition within descending pathways

**Peripheral mechanism:**

**Alteration in sodium channel distribution and expression:**

Inherited pain disorders due to mutated sodium channels (Yang Y et al., 2004[110], Cox JJ et al., 2006[111]) and polymorphisms of sodium channel have impact on the level of pain in patients, indicating that differences in sodium channel distribution might explain variability in pain seen in patients with DSPN (Reimann F et al., 2010[112]). In damaged peripheral nerves, the normal distribution of sodium channels along a nerve is altered due to neuroma. There occurs an ***accumulation of sodium channels*** at or around the site of injury resulting in "ectopic" activity. The potassium channels acts as molecular brakes of excitable cells, thereby modulating neuronal hyper excitability.

**Alteration in voltage-gated $Ca^{2+}$ channel distribution and expression:**

Voltage gated calcium channels serve to release transmitters, glutamate and substance P in pain fibres. Peripheral nerve injury leads to ***upregulation of α2δ subunits*** of voltage gated calcium channels, resulting in increased release of the neuro transmitters at the spinal cord (Bauer CS et al., 2009[113]).

**Central mechanisms:**

Studies concerned with changes in central nervous system following peripheral nerve injury have demonstrated ***increase in thalamic vascularity*** (Selvarajah D et al., 2011[114]), ***abnormal firing of thalamic neurons*** (Lenz FA et al., 1987[115]) and ***variations in concentration of metabolites in the thalamus*** (Pattany PM et al., 2002[116]). Aδ and C fibres that mediate pain terminate in the superficial laminae of the dorsal horn. These fibres finally project to limbic cortex, thalamus, nucleus tractus solitarius, periaqueductal gray, lateral parabrachial nucleus and the medullary reticular formation. Following neuropathy, the threshold of some of the neurons get reduced and results in hyper excitability of neurons. This in turn leads to increased response to all subsequent inputs, innocuous and noxious, explained by ***expanded receptive fields*** and augmented output to higher levels of the brain, termed central sensitization. Diabetic neuropathic pain is worse at night due to nocturnal changes in these central pain processing areas.

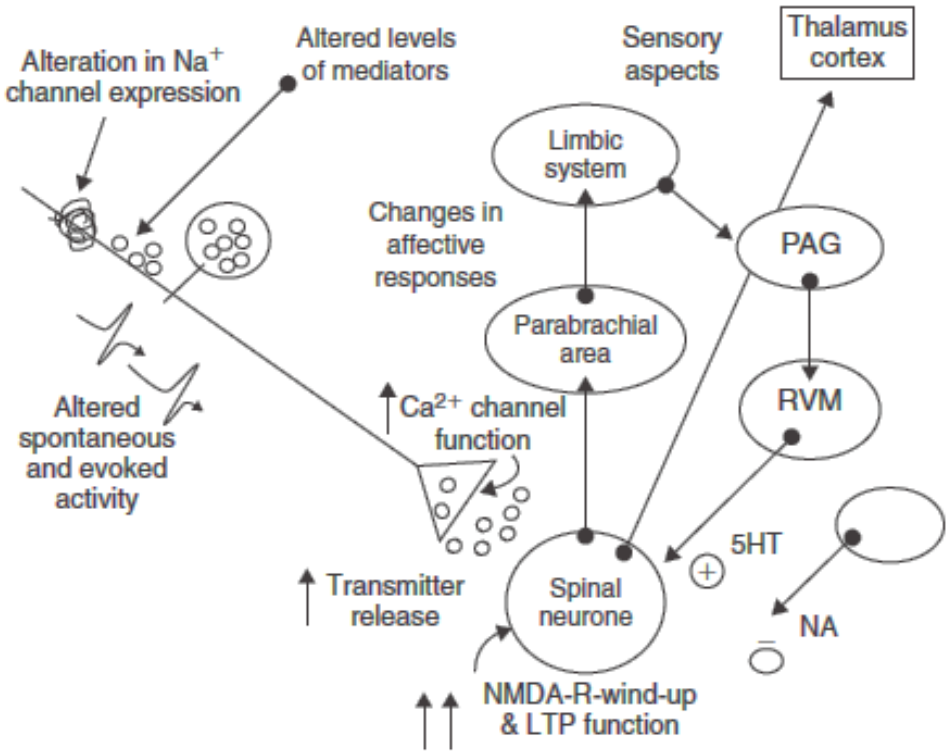



**Fig.13. Pathways of pain.** NA- Nor adrenaline, NMDA- N-Methyl D-Aspartate, PAG- Peri Aqueductal gray, LTP- Long Term Potentiation, RVM- Rostro Ventral Medulla

**Present treatment available for diabetic neuropathy:**

The effectiveness of using tricyclic antidepressants in DSPN has been documented by various randomized controlled trials (Max MB et al., 1992[117], Finnerup NB et al., 2005[118], Tesfaye S, 2009[119]).

**Tricyclic anti-depressants (TCA):**

Small doses (10 mg) of either amitriptyline or imipramine are usually prescribed at night. Use of TCA treatment of diabetic neuropathy has become limited because of the ***undesirable anticholinergic side effects*** like dryness of

mouth, sedation, sweating and dizziness. Recent studies have proved that they also increase the risk of ***sudden cardiac death*** in diabetic patients with cardiovascular disease (Ray WA et al., 2004)[120].

**Serotonin Noradrenalin Re-uptake Inhibitors (SNRI):**

Duloxetine, a Serotonin Noradrenalin Re-uptake Inhibitors (SNRI) act by increasing the synaptic availability of 5-hydroxytryptamine (5-HT) and noradrenaline in the descending pathways that inhibit pain impulses. Duloxetine at doses of 60 to 120 mg/day has been proved effective in treatment of painful diabetic neuropathy Goldstein DJ et al., 2005[121], Raskin J et al., 2005[122], Wernicke JF et al., 2006[123]). The most frequent side effects of duloxetine, usually mild and transient, are nausea, dry mouth, somnolence, dizziness, constipation, and reduced appetite.

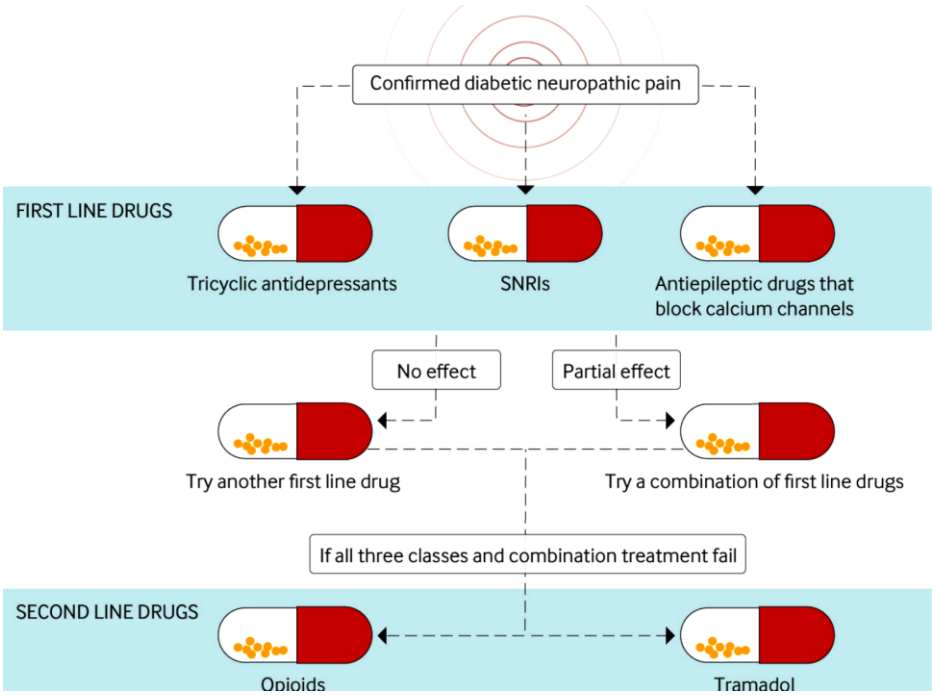



**Fig.14. Pharmacological treatment of diabetic neuropathy**

**Gabapentin and pregabalin:**

Gabapentin and pregabalin act by binding to the ***α-2-δ subunit of calcium channel*** and reducing calcium flux and thereby limiting the neurotransmitter release from the hyper excited neurons.

## PRINCIPLES OF SENSORY NERVE CONDUCTION

**Nerve Action Potential:**

Application of electrical stimulus to a nerve fibre, either sensory or motor, leads to opening of voltage-gated $Na^+$ channels in the membrane of nerve fibre and influx of sodium which in turn produces a local depolarization of the membrane. On attaining the threshold for depolarization, a self-sustaining action potential is produced at that site and it propagates along the nerve fibre by depolarizing the membrane in a sequence along both the directions from the point of stimulation.

**Sensory Nerve Action Potential (SNAP):**

The action potential generates an electrical field which is measured at a small distance from the nerve fibre. In a nerve trunk, when many nerve fibre action potentials propagate simultaneously, their electrical fields in the surrounding region get summated and this region is termed as volume conductor. SNAP represents the sum total of action potentials recorded from all nerve fibres present in the volume conductor. Sensory nerve conduction studies involve stimulation of the nerve and direct recording of the sensory nerve action potential

(SNAP) by surface electrodes placed over the nerve. The magnitude of the response falls as a function of the square of distance between the site of stimulus application and the recording electrode.

**Recording of SNAP:**

Sensory nerve conduction may be performed antidromically or orthodromically. The stimulation at distal portion of the nerve and recording the sensory nerve action potential proximally along the nerve is called orthodromic conduction. The stimulation at proximal part of the nerve and recording the sensory nerve action potential at the distal part of the nerve is known as antidromic conduction. In orthodromic conduction, the impulses travel in the same direction as that of sensory impulses, whereas in antidromic condition, the impulses travel in the opposite direction as that of sensory impulses (Binnie CD et al., 2004[124]). Antidromic technique is preferred over orthodromic technique.

**Patient Position:** Comfortable, resting posture.

**Electrode Placement:**

**Active (recording) electrode and reference electrodes:** Placed over the segment of nerve which is being studied and active electrode is placed distal to reference electrode

**Ground electrode:** Placed over a bony prominence amid stimulating and active (recording) electrodes

**Electrostimulation:** Stimulation is given percutaneously using surface electrodes along the course of the nerve segment under study.

Cathode (negative pole) of the stimulating electrode must be placed towards the active (recording) electrode.

**Sensory nerve action potential (SNAP)**

SNAP is a tri phasic potential. It has an initial and terminal positivity separated by a negative deflection. The negative deflection indicates the time of impulse arrival beneath the active electrode.

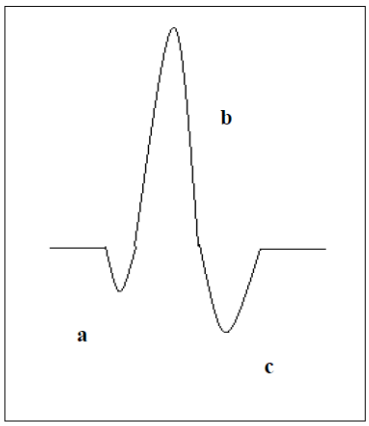



**Fig.15. Sensory Nerve Action Potential**- Triphasic potential with initial and terminal positive peaks (a & c) and middle negative peak (b)

**Parameters that are measured in sensory nerve conduction study:**

1. Amplitude
2. Latency
3. Nerve conduction velocity
4. Duration

**Amplitude:**

The amplitude of a sensory response in microvolts is "measured from the peak of the negative phase to the peak of the positive phase of the sensory response". It is associated with the number of axons that are viable. The SNAP amplitude is the algebraic sum of the electrical activity recorded at the two electrodes. If active and reference electrodes are placed close to each other, both the electrodes become active and this may distort the waveform and decrease the amplitude of action potential (Willbourn AJ 1994[125]). Hence, distance between active and reference electrode should be at least 3 cm, in order to clear the potential at active electrode before the activity begins at the reference electrode. SNAP amplitude usually varies from 5 to 200 µV.

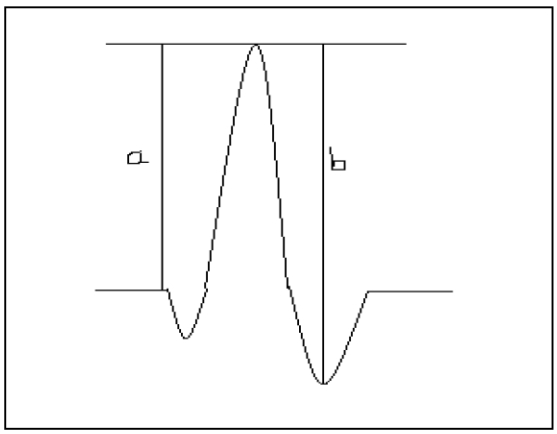



**Fig.16. Measurement of SNAP amplitude.** a- base to peak amplitude and b- peak to peak amplitude.

**Latency:**

Time lapsed from the stimulus application to the onset of response is known as latency. Latency is related to the propagation of impulse along the

nerve. Latency for sensory nerve conduction is calculated from the onset of stimulus to peak of the response and is expressed in millivolts. The onset latency of SNAP is representative of the nerve conduction time and hence one site of stimulation is sufficient for sensory nerve conduction studies.

Conduction latency time (milliseconds) for a sensory response is "measured from the stimulus artifact to the peak of the negative phase of the sensory response".

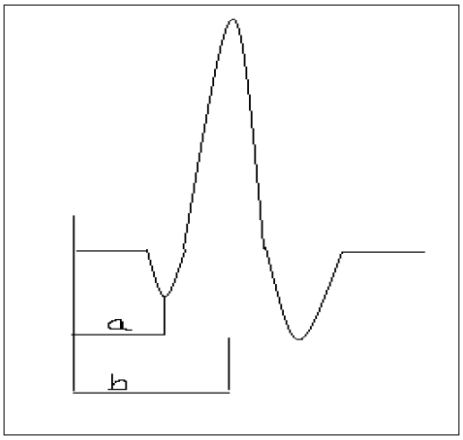



**Fig.17. Measurement of latency of SNAP.** a- onset latency and b-peak latency

**3. Duration:**

Duration is measured "from the initial positive peak to the junction between baseline and the descending wave". The duration (milliseconds) of a motor or sensory response is measured "from the initial deflection of the negative phase of the response from the iso-electric baseline to the return of the positive phase of the response to the iso-electric baseline".

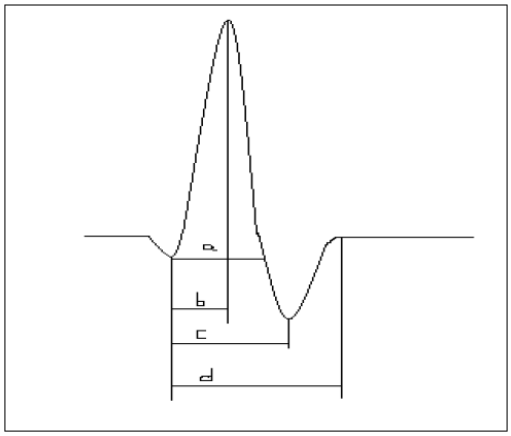



**Fig.18. Measurement of duration of SNAP.** a - onset to intersection between the descending phase and base line, b – onset to negative peak, c – onset to positive peak and d – onset to return to base line.

**4. Sensory Nerve Conduction Velocity (SNCV):**

SNCV is the ratio of distance (in millimetre) between the centre of stimulating electrode and recording electrode to the latency (in milliseconds).

$$\text{SNCV (m/s)} = \frac{\text{Distance between stimulating and recording electrode}}{\text{Latency}}$$

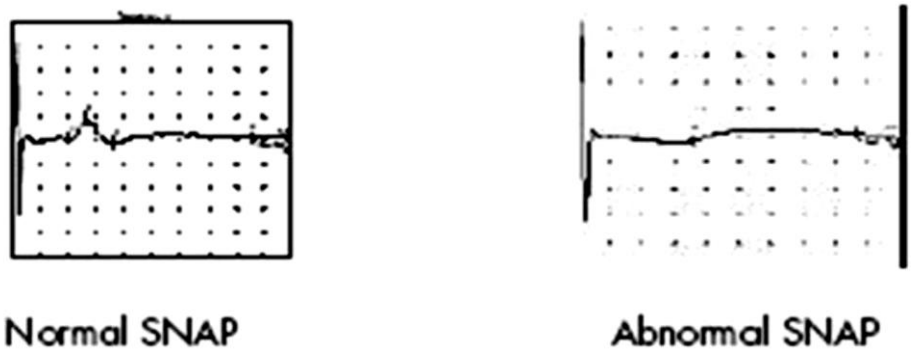



**Fig.19. Normal and abnormal Sensory Nerve Action Potential**

**Clinical applications of nerve conduction study:**

Nerve conduction study helps in detecting the nerve damage and determining the pattern of nerve damage. Nerve damage may be either axonal degeneration or demyelination. Usually, a mixed pattern with both axonal loss and demyelination occurs. Axonal loss may lead to slowing of conduction due to secondary demyelination and severe demyelination may lead to secondary axonal loss.

**Axonal degeneration**

Axonal loss usually follows neuronal cell death or injury to distal segment. This leads to interruption of impulse transmission at the most distal part of the axon, termed as a 'dying back phenomenon' (Cavanagh JB., 1964[126]). Pathological changes seen in axonal loss are axolemmal and myelin sheath disruption, loss of cytoskeletal elements without affecting the Schwann cells (William W. Campbell., 1999[127]). Electrophysiological changes seen in pure axonal loss are decrease in the amplitude of action potential with preservation of conduction velocity as the axons which survive conduct at normal velocity (Binnie CD et al., 2004[124]).

**Demyelination:**

Demyelination primarily affects either the myelin sheath or Schwann cell and the axon is intact. Demyelination may be para nodal or Segmental demyelination affecting the whole segment of myelin sheath.

Electrophysiological changes seen in demyelination are conduction block in particular node with normal distal nerve conduction in the case of para nodal demyelination and decrease in conduction velocity with normal amplitude of action potentials in segmental demyelination (Bostock H., 1993[128]).

**Nerve conduction study in DPN:**

Currently, Nerve conduction studies (NCS) remain the most sensitive and specific method for detection of Diabetic Peripheral Neuropathy (Perkins BA, 2001[129]). Nerve conduction studies provide a quantitative confirmation of Diabetic Peripheral Neuropathy (Boulton AJ et al., 2005[130]). Widespread use of Nerve Conduction Study has helped in early diagnosis and superior outcomes (Perkins BA et al., 2003[131]). Sensory nerve conduction is most commonly used for finding the response to a treatment (Sisken BF et al., 1993[23]). A study by ***Y.L. Lo et al***., [132] has compared the superficial peroneal and sural NCS for detecting peripheral neuropathy and has found superior results with Superficial Peroneal Nerve.

**History of using magnets in treating diseases:**

Since 15th century, various biological effects of low-level magnetic fields are being studied. Swiss physician, Paracelsus, during early 16th century, introduced the novel idea of using magnets in treating diseases like epilepsy, diarrhoea and haemorrhage (Mourino, M., 1991[133]). Later during the middle of 18th century, magnetic therapy became more popular, when an Austrian doctor,

Franz Mesmer, started his magnetic healing salon in Paris. Dr.Thatcher had remarked that "magnetism properly applied will cure every curable disease no matter what the cause is." (Thatcher, C. 1886[134]).

**Electromagnetism:**

Electromagnetism was first discovered by the 'English physicist Michael Faraday', during 18th century. He found that 'on allowing an electric current to flow through a coil, it generated a magnetic field and in other hand, a varying magnetic field produced an electric voltage'. The magnetic field must be changing in order to produce an electric effect. Biological effects of Pulsed Electromagnetic Fields are assumed to be because of electrical rather than magnetic forces.

***Voltage generated by magnetism in a tissue is given by the equation:***

$$\mathbf{V = n \times a \times dB/dt}$$

'V' = 'Voltage'

'n' = 'Number of turns in the electromagnetic coil'

'a' = 'Area of the loop'

'dB/dt' = 'Rate of change of magnetic field with respect to time'

'B denotes the strength of the magnetic field (in Teslas)'

From this equation, it can be deduced that a static magnetic field does not produce any electrical voltage, as the ***'dB/dt component'*** of the equation becomes zero.

The magnetic field generated by the movement of electrons are a field of virtual photons that initiate force lines. This magnetic field make the particles with an electric charge such as ions, to move. This force is known as a Lorentz force.

**Bio electromagnetics:**

Bio electromagnetics deals with the study of interaction between the biological system and non-ionising electromagnetic fields. Any modality that uses electricity and produces both an electric and a magnetic field is called electromagnetic modality. Movement of electrons (Flow of electricity) and the resultant 'coupled magnetic field' that is produced by the movement of electrons altogether is named as Electromagnetic radiation. Specific biological responses are generated by specific types of ELF Electromagnetic Fields based on certain parameters (Magnitude, frequency, wave form) (B.Rubik, 1997[135]). A subsection of Extremely Low Frequency Electromagnetic Fields, called Pulsed Electro Magnetic Field (PEMF) includes frequencies at the lower end of the electromagnetic spectrum, say from 6 Hz to 500 Hz (C.A.Bassett, 1989[136]). Voltages ranging from 10 μV and 1 mV/cm produced with a frequency of < 1 Hz to 100 Hz result in an electric field, whether arising endogenously or produced exogenously by inductive coupling, appropriately configured, pulsing magnetic fields, have the potential to affect the functioning of various cells (McLeod KJ et al., 1990[137]).

**Mechanism of action of PEMF:**

The main effect of PEMF is due to induced electric field component rather than magnetic field component (C.A.Bassett, 1989[136], Bassett CAL, 1993[138], Pilla AA et al., 1992[139]). Electromagnetic modalities generate an electrical field using direct current running between the electrodes, leading to electrons mobility which is expected to influence the excitable cells by making ions within the excitable cells to move towards the respective electrodes and thereby, affecting physiology of the cell. Negative ions like chlorine ($Cl^-$) are attracted towards the positive pole (anode), and positive ions like Sodium ($Na^+$) move towards the negative pole (cathode). The levels of stimulation achieved with stimulating excitable cells may be sub sensory, sensory, motor and noxious (Panagopoulos DJ et al., 2002[140]).

If we use a simple direct current wherein the electrons are flowing only in a single direction, there would be accumulation of like-charge ions in one region. This would increase the concentrations of hydrochloric acid and sodium hydroxide, thereby affecting the local pH which may in turn lead to pain and cellular damage. When we use a current which is alternating and bi-directional in nature, the disproportionate accumulation of ions near an electrode can be prevented.

Panagopoulos et al. put forward a hypothesis stating that 'externally applied electromagnetic field' makes the ions to vibrate (Ganesan K et al., 2009[141]). This vibration, upon reaching a 'critical point', generates a 'false signal' to the voltage gated channels present in the membranes of eukaryotic

cells. This false signal, forces the voltage gated channel to either 'open or close' affecting the cellular physiology. Panagopoulos et al. has also described the similarity in effects of the oscillating electric and magnetic fields on the free ions and subsequently the 'voltage gated ion channels'. The theory put forward by Panagopoulos et al. states that, the amplitude of the "ion's forced vibration" is inversely related to the frequency, and hence electromagnetic fields in the lower frequency ranges have the likely to be more bioactive.

**PEMF acts through two mechanisms:**

a. By stabilising cytosolic calcium ($Ca^{2+}$)

b. Restoring equilibrium between free radicals and antioxidants

**Stabilisation of cytosolic calcium ions:**

Electro Magnetic Field signals act as a first messenger that modulates Calmodulin (CaM) dependent pathways (A.A. Pilla, 2007[142]) which is normally activated by increased cytoplasmic free calcium ions ($Ca^{2+}$) concentration following insult, injury and stress (M.J. Berridge et al., 2003[143]). Most important pathway that rapidly responds to physical, chemical insults in various tissues is the CaM-dependent nitric oxide (NO) signalling pathway. Nitric oxide, a gaseous free radical, is capable of diffusing locally across membranes and organelles and exerts effect on molecular targets located within 200 μm (N.M. Tsoukias, 2008[144]). Nitric Oxide activates soluble guanylyl cyclase (sGC). Guanylyl cyclase in turn catalyses synthesis of cyclic guanosine monophosphate

(cGMP) (Cho HJ et al., 1009[145]). The CaM/NO/cGMP signalling pathway is capable of modulating peripheral and cardiac blood flow during physiological states and inflammation (D.S. Bredt, et al., 1990[146]), regulating the interleukin-1beta (IL-1β) production (K. Ren et al., 2009[147]) and growth factors like vascular endothelial growth factor (VEGF) production and fibroblast growth factor (FGF-2) (S. Werner et al., 2003[148]).

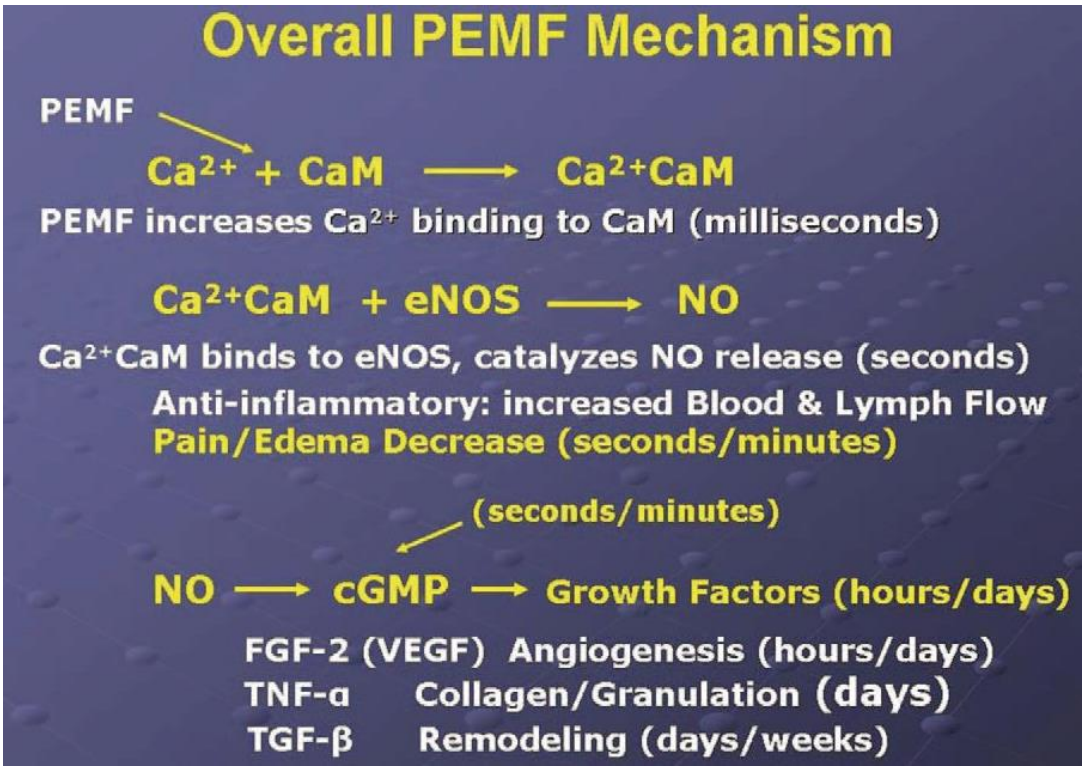



**Fig.20. Mechanism of action of PEMF**

Following an injury, e.g., a bone fracture or a surgical incision, repair commences with an inflammatory stage during which the pro-inflammatory cytokine IL-1β is rapidly released. This signalling pathway can also activate adenylyl cyclase (AC) that catalyses the formation of cyclic adenosine monophosphate (cAMP).

Recently, it has been proposed that CaM/nNOS/NO signalling may regulate the neuronal cell differentiation (Soo-Jin Oh1 et al., 2010[149]). Besides increasing cell metabolism, PEMF has the ability to ameliorate inflammatory effects by reducing inflammatory cytokines.

**Restoring equilibrium between free radicals and antioxidants:**

Almost all the chronic diseases result from an imbalance in homeostasis between the free radicals and antioxidants. Together, the free radicals and antioxidants, are essential for processes occurring within the cell, for instance, cellular respiration and immunity. Any discrepancy can result in death of cell and tissue, damage to DNA and degradation of protein and fat. As suggested by ***Gordon***[150], PEMF also has another essential effect, the ability of the magnetic fields to restore "equilibrium in ROS (free radicals)/antioxidants".

Gordon elaborates that both reactive oxygen species (ROS) free radicals like superoxide anion ($O_2^-$) and hydroxyl anion ($OH^-$) are paramagnetic in nature and they will be affected by a magnetic field (analogous to the effect on ions: $K^+$, $Na^+$, $Cl^-$, $Ca^{2+}$). These para magnetic species are attracted by the electromagnetic field and undergo dipole alignment in a magnetic field (Zumdahl S., 1992[151]). It is assumed to increase the homeostasis between ROS and antioxidants.

**Effect of PEMF on various cells:**

| Study | PEMF parameters | Inference from the study |
|---|---|---|
| Richards TL et al[152] | 75 Hz, 2.3 mT | Increased number of chondrocytes |
| Mattei MD et al.[153] | 15 Hz, 0.1 mT | Increased proliferation of osteoblasts |
| Gomez Ochoa et al.[154] | 75 Hz, 0.2mT - 3.5mT | Saturation of adenosine receptors causing decreased inflammatory cytokine cascade |
| Farndale R et al.[155] | 50 Hz, 2.25 mT | Significant increase in the Pro inflammatory cytokine levels, IL-1 $\beta$ and TNF- $\alpha$ |
| Delle Monache S et al.[156] | 15 Hz, 4.8 ms pulse | Reduction in cAMP levels leads to increased collagen cell proliferation |
| Pipitone N et al.[157] | 50 Hz, 1mT | Increased endothelial cells proliferation resulting in angiogenesis |
| Fini M et al.[158] | 6 weeks of once-daily stimulation | PEMF enhances osteo integration of hydroxyapatite into cancellous bone |

**Studies concerned with effect of PEMF on various clinical conditions:**

**1. Musculoskeletal disorders:**

***a. Non-union of bone fractures***

- ***Bassett et al.***[159] reported radiographic evidence of osteogenesis in 25 of 29 surgically resistant non-union patients after 75 Hz PEMF for one month

- ***Heckman et al.***[160] demonstrated enhancement of bone healing after 3 months of PEMF therapy in 149 non-union patients with 85% success rate
- ***Frykman et al.***[161] showed healing of non-union of scaphoid fractures in 44 patients with 80% success rate using PEMF as an alternative to long arm cast

b. ***Rheumatoid arthritis:*** *Ganguly et al.*[162] evaluated the effectiveness of PEMF in 35 patients with rheumatoid arthritis. Results showed an earlier improvement in pain, tenderness, swelling, physical disability and joint spasm and deformity in seronegative rheumatoid arthritis patients compared to seropositive individuals.

c. ***Osteoarthritis***:

- ***Piptone et al.***[163] used 7.8 Hz (morning) and 3 Hz (evening) of PEMF in patients with osteoarthritis knee. This study reported significant improvements in pain, physical disability and stiffness scores after PEMF therapy compared to their baseline scores
- ***Jacobson et al.***[164] demonstrated reduction of pain in patients with osteoarthritis knee using low amplitude and extremely low frequency magnetic field therapy.

**2. Soft tissue regeneration**

***a. Venous leg ulcers***

- ***Ieran et al.***[165] did a double blind study for the treatment of patients with venous leg ulcers with 75 Hz PEMF for 90 days. They showed a

significant success rate of 66% in patients exposed to PEMF and follow up of these patients for 1 year post PEMF therapy revealed a decrease in recurrence of ulcers.

- ***Stiller et al.***[166] did a multicentre, prospective, randomised double-blind study for treatment of recalcitrant venous ulcers using PEMF for 12 weeks. The results showed a significant (47.7%) decrease in wound depth and only 15% increase in healthy granulation tissue.

**3. Neurological disorders**

***a. Median-ulnar nerve injury:*** *Wilson et al.*[167] assessed the median-ulnar nerve regeneration in the forelimb of 132 rats administering PEMF for 2 months. The results indicated rapid regeneration of the median-ulnar nerve

***b. Peroneal nerve injury:*** *Raji et al.*[168] used male Lewis rats to assess the effect of PEMF on peroneal nerve injury. The results showed a significant increase in number of nerve fibres indicating nerve regeneration and improvement in toe spreading reflex.

***c. Sciatic nerve injury:*** *Kanje et al.*[169] demonstrated a significant sciatic nerve regeneration following crush injury to male and female rats with PEMF of 2 Hz exposed for 1 hour daily.

***d. Multiple sclerosis***: *Richards et al.*[170] evaluated the effect of PEMF (4-13 Hz, 10-20 hours/ day for 2 months) in patients with multiple sclerosis. The results revealed improvement in performance scales and increase in alpha EEG during language tasks.

e. ***Tinnitus:*** *Roland et al.*[171] performed a randomized double-blind study for the treatment of tinnitus. The results showed a significant reduction of sensation level (45%) and improvement in symptoms.

f. ***Cerebral ischemia***: *Grant et al.*[172] demonstrated a significant reduction (65%) in cortical oedema (neocortex and neostriatum) in a rabbit model with focal cerebral ischaemia.

**4. Coronary disorders**

- ***DiCarlo et al.***[173] did a study on cardiac anoxia damage in chick embryo using 60 Hz of PEMF therapy. The results showed a significant (68.7%) increase in survival rates following PEMF therapy.
- ***Albertini et al.***[174] showed a reduction in necrotic region of myocardial infarct following 60 Hz PEMF therapy

**Studies concerned with effect of PEMF on diabetic neuropathy:**

***Weintraub et al.***[175] studied the effectiveness of repeated and cumulative effect of PEMF in reduction of neuropathic pain, sleep disturbance and nerve regeneration in 225 diabetic neuropathy patients. The study subjects were exposed to PEMF or sham for 2 hours/ day for 3 months. The study concluded that 31 mT magnetic field exposure did not reduce neuropathic pain however, Epidermal Nerve Fibre Density (ENFD) was found to increase ($p = 0.04$) and itching sensation was reduced ($p = 0.48$).

***Vinay Graak et al.***[176] compared the effect of PEMF of frequencies 600 Hz and 800 Hz in diabetic neuropathy patients. It was a randomized double

blinded controlled trial that included 30 diabetic neuropathy patients, of which 10 were exposed to 600 Hz PEMF, 10 were exposed to 800 Hz PEMF and the remaining 10 served as a control. Significant reduction in pain and improvement in distal latency and Nerve Conduction velocity (NCV) ($P<0.05$) was observed in groups that were given PEMF in comparison to the control group.

***Tao Leil et al.***[177] evaluated the therapeutic potential of PEMF in relieving symptoms of diabetic peripheral neuropathy in experimental streptozotocin (STZ)-induced diabetic adult male Sprague–Dawley rats. The exposure group received 8 hours of PEMF for 7 weeks. The study revealed that PEMF stimulation mitigated the abnormalities in STZ-treated rats with DPN. There was an increase in hind paw withdrawal threshold, reduced demyelination and axon enlargement, less immunostaining of VEGF in sciatic nerve in exposure group in comparison with non-exposure group.

***Ismail Gunay et al.***[178] evaluated the effect of PEMF on regenerating peripheral nerves conduction characteristics of injured sciatic nerves in rats by using sucrose-gap recording technique. The study demonstrated the absence of abnormal hyperpolarizing after potentials and delayed depolarisation (found in injured nerves) following PEMF application. PEMF treatment for 38 days after injury produced significant differences in the conduction of Compound Action Potentials ($p<0.05$).

***Musaev et al.***[179] compared the effectiveness of 10 Hz and 100 Hz PEMF for Diabetic neuropathy and reported that 10 Hz is more effective in comparison

to 100 Hz for treating sensory deficits in diabetic neuropathy. The drawback of this study is that it does not include any control group.

***Wrobel et al.***[180] assessed influence of low frequency magnetic field on intensity of pain, sleep, quality of life and glycaemic control in painful diabetic polyneuropathy patients. This was a randomized, double-blinded placebo-controlled study that included 61 patients. The study group with 32 patients were exposed to low frequency magnetic fields for 20 min a day, five days a week for 3 weeks. The pain reduction did not differ significantly between the study and the control group.

***Webb et al.***[181] administered PEMF of 12 Hz frequency for 30 minutes and in foot of diabetes mellitus patients and had found an increase in peripheral blood flow and skin microcirculation.

***Cieslar, G et al.*** [182] employed sinusoidal magnetic fields for 12 minutes a day to treat diabetic neuropathy. The study demonstrated reduction in pain and paraesthesia and improvement in vibration sensation and muscular strength in 85% of patients in comparison to controls.

# AIM AND

# OBJECTIVES

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**Aim:**

To determine whether low frequency Pulsed Electro Magnetic Field therapy (PEMF) can reduce neuropathic pain, influence nerve regeneration and improve vascularity of foot in type-2 Diabetes mellitus patients with painful sensory polyneuropathy

**Objectives:**

1. To administer Pulsed Electro Magnetic Field therapy to Type-2 diabetes mellitus patients with painful diabetic sensory neuropathy
2. To do nerve conduction study in superficial peroneal nerve and to assess and compare the distal latency, amplitude and nerve conduction velocity before and after Pulsed Electro Magnetic Field therapy
3. To assess and compare the neuropathic pain before and after the Pulsed Electro Magnetic Field therapy using Visual Analog Scale
4. To assess and compare the vibration Perception Threshold using Biothesiometer before and after Pulsed Electro Magnetic Field therapy
5. To assess and compare the vascularity in foot before and after Pulsed Electro Magnetic Field therapy using Tc-99 MDP 3 phase bone scan
6. To assess and compare the erythrocyte Super Oxide Dismutase levels before and after the Pulsed Electro Magnetic Field therapy

# MATERIALS AND METHODS

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The study was conducted during the period of June 2014 to May 2015 at the Institute of Physiology and Experimental Medicine, Madras Medical College and Institute of Diabetology, Rajiv Gandhi Government General Hospital after obtaining approval from Institutional Ethics Committee (IEC), Madras Medical College, Chennai-3.

**Selection of subjects:**

Thirty type 2 diabetes mellitus patients in the age group of 40 to 60 years with painful sensory polyneuropathy were selected from the Institute of Diabetology, Rajiv Gandhi Government General Hospital for the study.

**Inclusion criteria:**

Both men and women with the following were included in the study:

- Age group: Between 40 and 60 years
- Duration of type 2 diabetes mellitus: More than 10 years
- Suffering from painful sensory polyneuropathy assessed by a Questionnaire for Neuropathy Symptom Score (NSS) of Dyck and with a score of 1 to 4
- Good Glycemic control (HbA1c <7)

**Exclusion criteria:**

Patients with the following conditions were excluded from the study:

- Type-1 diabetes mellitus
- Painless sensory polyneuropathy
- Diabetic ulcers
- Nutritional deficiencies
- Hansen's disease
- Active Tuberculosis
- Coronary artery disease
- Deep Venous Thrombosis
- Malignancy
- Radiculopathy
- Pacemakers

**Methodology:**

Thirty patients in the age of 40 to 65 years with type-2 diabetes mellitus of more than ten years duration and Dyck's Neuropathy Symptom Score of 1-4 with painful diabetic neuropathy with good glycemic control participated in the study.

After obtaining informed consent, the participants of the study were subjected to nerve conduction study in superficial peroneal nerve using MEDICAID Physio-pac nerve conduction study machine, Vibration Perception Threshold (VPT) was assessed by Biothesiometer, pain was assessed using

**Questionnaire for Neuropathy Symptom Score (NSS) of Dyck**

**Name of the patient:**

**Age/ sex:**

**OP no:**

The questions should be answered 'yes' (positive: 1 point) if a symptom occurred more times a week during the last 2 weeks or 'no' (negative: No point) if it did not.

1. Symptoms of unsteadiness in walking? **Yes/ No**
2. Do you have a burning, aching pain or tenderness of your legs or feet? **Yes/ No**
3. Do you have pricking sensations at your legs and feet? **Yes/ No**
4. Do you have places of numbness on your legs or feet? **Yes/ No**

**Neuropathic Symptom score of Dyck:** 1 2 3 4

**Maximum score:** 4 points

**0 points**- PNP absent

**1-4 points** - PNP present (PNP = Polyneuropathy)

Visual Analog Scale (VAS), blood samples were taken for estimating erythrocyte Super Oxide Dismutase (SOD) levels and the vascularity of foot was assessed by Tc-99 MDP 3 phase bone scan.

The participants were subjected to Pulsed Electro Magnetic Field Therapy using instrument designed by ***Madras Institute of Magnetobiology*** delivering ***1500 nTesla (10Hz frequency with square wave configuration),*** at the Institute of Diabetology, Madras Medical College and Rajiv Gandhi Government General Hospital for ***60 minutes/ day for 21 days with a break after every 6 days.***

After the Pulsed Electro Magnetic Field therapy, the subjects again underwent a nerve conduction study in superficial peroneal nerve, vibration perception threshold was assessed, pain was assessed using visual analog score, blood samples were taken for estimating erythrocyte Super Oxide Dismutase (SOD) levels and the vascularity of foot was assessed by Tc-99 MDP 3 phase bone scan.

**STUDY DESIGN :** Case Control Study

**TYPE OF STUDY :** Observational study (Prospective)

**PLACE OF STUDY:** Institute of Physiology & Experimental Medicine, Madras Medical College, Chennai-3.

Institute of Diabetology, Rajiv Gandhi Government General Hospital, Chennai-3.

**Pulsed Electro Magnetic Field (PEMF) therapy:**

PEMF was delivered to the patients by the instrument-PULSATRON from the ***Institute of Magnetobiology, Anna Nagar.***

PEMF therapy is a technique of "sweeping" or "bathing" the entire body or the region of interest in a highly homogeneous pulsing magnetic field having a very low intensity and extremely low frequency. PEMF's are produced within special controlled magnetic field enclosures (Coil systems) by allowing measured amount of electric current to flow through them. The amplitude, frequency and waveform of the electric current flowing through the coil can be regulated using signal generators. Region of interest that has to be subjected to the PEMFs produced within the coil system must be positioned within the area of most uniform magnetic flux density for the preset duration of exposure. PEMF therapy is totally non-invasive and does not require administration of any medications. Until recently, no side effects have been reported on the very low intensity and extremely low frequency magnetic field spectrum.

**Magnetic field enclosure:**

The coil assemblies, designed and constructed at the Madras Institute of Magnetobiology (S.Shivakumar et al., 2013[183]) are standardized with magnetometers of high precision and ammeters in the Magnetic Standardization Lab of the Institute.

A Four-member coil system, derived from the fundamental equations of a ***Fanselau-Braunbeck coil system*** is used. It is made up of two set circular coils

array, of which the inner two are of larger diameter and the outer two are of smaller diameter. The coils are mounted co-axially and in a co-planar fashion so that it forms an enclosure wherein the arrangement of the coils has a unique ratio of distance to radius of the coils. This arrangement produces a highly uniform magnetic field to be produced within the enclosure for a given amount of current (usually in milli-Ampere) along the axis of the coil system. The magnetic field so generated is homogeneous such that deviation in the field uniformity within the two central rings is found to be 1/5000.

To regulate the magnetic field produced, a hardware which consists of a standard ***signal generator*** along with a meter assembly is used. The signal generator delivers the current to the coil assembly. It operates at a voltage of 110/220V and frequency of 50 Hz at the primary end. At the secondary end, the signal generator deliver currents (in mA ranging from 10 to 100) at desired waveforms (either sine, square or ramp) in different frequencies (varying between 0.1 Hz to 1000 Hz at voltages 0.2 to 20 volts). Class 2 type (pre-calibrated) milli-ammeter is used to measure the current which is delivered by the signal generator to the coil.

**Exposure protocol:**

**Parameters used in this study:**

- Frequency: 10 Hz
- Waveform: Square wave
- Duration: 60 minutes/day for 21 days (Break after every 6 days)

**Photograph: 1** PULSATRON- Magnetic field enclosure and signal generator hardware

PULSATRON- Magnetic field enclosure from Madras Institute of Magnetobiology


Signal generator hardware from Madras Institute of Magnetobiology

The patients were exposed to pulsed electromagnetic fields according to the following pre-determined parameters.

| Duration of therapy | 30 min/day | 45 min/day | 60 min/day | 90 min/day | 120 min/day |
|---|---|---|---|---|---|
| PMF parameters | for 30 days max | for 30 days max | for 45 days max | for 21 days | for 21 days |
| 1 Hz, Sine wave<br>Therapy Program 1 | Foot ulcer grade-1 as per wagner scale | Foot ulcer grade-2 as per wagner scale | Fractures | Simple burns | |
| 10 Hz, Sine wave<br>Therapy Program 2 | | sports injuries | pain management single joint | Pain management with Multiple joint involvement moderate | severe |
| 10 Hz, square wave<br>Therapy Program 3 | simple migraine | mild depression (seasonally triggered)<br>mild hypertension | Muscle Spasticity<br>diabetic neuropathy<br>stroke management<br>Cerebral palsy<br>Parkinsons' disease | | |

**Fig.21. PEMF Exposure protocol for diabetic neuropathy designed by Madras Institute of Magnetobiology**

**Nerve conduction study (NCS):**

Nerve conduction study in the superficial peroneal nerve was performed by using MEDICAID Physio-Pac machine.

Nerve conduction studies are the technical procedures that objectively assess the functional status of peripheral nerves. NCS comprises of recording, displaying, quantifying and interpreting the action potentials arising from peripheral nerves. These action potentials are interpreted as waveform (Misra VK et al., 2006[184]). In this process, biological signals are detected, amplified and filtered to put them into usable form.

**Photograph.2.** Administration of PEMF to a patient

## HARDWARE DESCRIPTION

The electro diagnostic instrument consists of the following elements:

- Electrodes
- Amplifier
- Filters
- Averager
- Display
- Stimulator

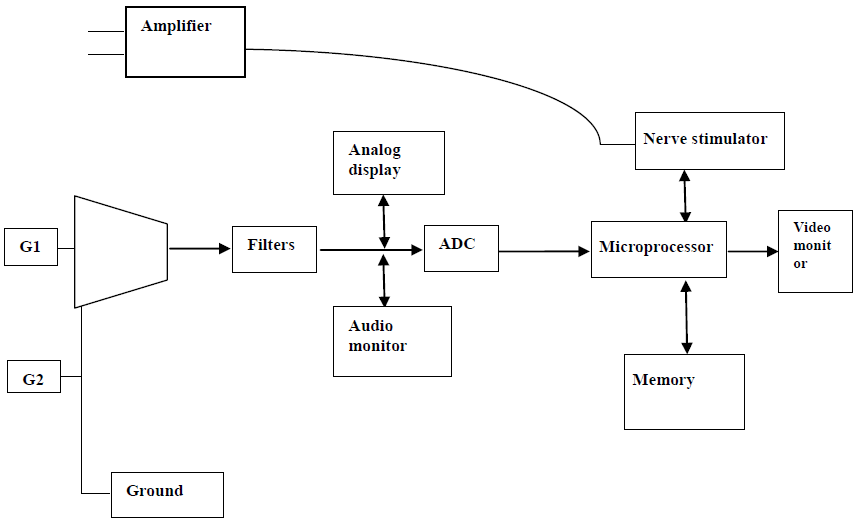



**Fig.22. Schematic diagram showing major components of electro diagnostic equipment.** ADC- Analog to digital converter.

## ELECTRODES

Nerve conduction study involves use of three electrodes namely, active, reference, and ground electrodes. The potential difference between the active and reference electrodes is taken as action potential and the ground electrode functions as a zero voltage reference point.

Metals and alloys like stainless steel, platinum, nickel, chromium, silver and gold are used for making electrodes. The electrodes are of two types, surface electrode and needle electrode. Surface electrodes are commonly employed in nerve conduction study. The surface electrode may be of cup, disc, or ring shape of which the disc electrodes are commonly used in nerve conduction studies. Before placing the electrode, the skin must be cleansed thoroughly. Electrode paste or jelly may be used in fixing the electrode.

**AMPLIFIER**

Amplitude of biological signals are very small and amplification is necessary before processing. Transistors made up of semiconductors are used for amplification. The current flow in a transistor, starts from the emitter (E), passes through the base (B) to reach the collector (C). The process of amplification occurs at the emitter-collector circuit.

Intrinsic impedance of the electrode and the impedance of electrode-skin interface, together known as electrode impedance decreases the amplitude of the action potential. To minimise this decrease in amplitude, the impedance of the amplifier is maintained higher than the impedance of the electrode. Impedance of the surface electrode connections should be less than 5kΩ.

**FILTERS**

Biological signals comprises of a mixture of different frequencies from multiple sources leading to a complex waveform (Barry Halliwell et al., 1999[185]).

Filter selectively limits the frequency domain of a signal. Important information from the biological signal of interest may be sometimes altered or removed by filtering. Hence, filtering should be done in such a way that the desired signal is not altered.

Filter settings are usually depend on the frequency range of the biological signal of interest. Frequency range transmitted through the filter is known as filter band pass. Frequency range beyond which the signal is rejected is the stop band. Low frequency filter or high pass filter eliminates the low frequency components from the signal and allows the high frequency components to pass through the filter. High frequency filter or low pass filter eliminates high frequency components of the signal and allows low frequency components to pass through the filter.

Filters may be analog filters or digital filters. A combination of resistors, capacitors and amplifiers is used by analog filters. Digital filters make use of computer for converting the signal into a digital form (Maccabee PJ et al., 1992[186]). Currently, a combination of analog and digital filtering is in use.

The following are filter settings for low and high frequency filters used in nerve conduction study:

| **Test** | **Low frequency filter (Hz)** | **High frequency filter (kHz)** |
|---|---|---|
| Motor nerve conduction | 2-5 | 10 |
| Sensory nerve conduction | 5-10 | 2-3 |

## AVERAGER

Extraction of very small signals that are buried in larger noise is done by the process known as averaging. Averaging makes the randomly occurring noise less conspicuous, at the same time, it makes the regularly recurring signal more conspicuous.

## DISPLAY

The process of presenting the signals for analysis is called display. In analog oscilloscope display, the signals are amplified, filtered and directly displayed. Analog display has an added advantage that the waveform details can be reproduced, but it cannot quantify the waveforms which can be done by digital display. An analog to digital converter (ADC) converting analog signal into digital approximation and digital processing techniques are required for digital displays. Sampling frequency of the ADC (how often sampling of signal is done) and the vertical resolution (how many discrete digital steps are required for quantifying the amplitude) determines the precision of the replication of analog signals. More accurate digital replica of the signal can be obtained with a high sampling frequency and high resolution. Nyquist frequency, twice the fastest frequency in the sample, is the minimal sampling frequency required for accurate reproduction of the frequency content of the sample. Distortion of the waveform produced by sampling below the Nyquist frequency is known as "aliasing effect". Number of bits determine the vertical resolution of ADC.

**Resolution of ADC = $2^n$ (n= number of bits)**

An 8-bit processor will quantize a signal into 256 amplitude levels, a 12-bit processor will divide a signal into 4096 discrete steps and hence provide better resolution. After digital processing, the tracing is displayed using digital to analog converter to change it back into analog signal.

## GAIN AND SWEEP SPEED

The gain or sensitivity and sweep speed affect the latency and duration measurement of action potential. Greater the display sensitivity, larger is the latency and duration of action potential. Increasing the sweep speed reduces the latency (Dumitru d et al., 1988[187]).

Nerve conduction studies employ the following settings:

| | **Gain settings** | **Sweep speed settings** |
|---|---|---|
| Sensory nerve conduction | 10 – 20 μV/ division | 1 – 2 msec/division |
| Motor nerve conduction | 2 – 10 mV/ division | 2 – 5 msec/division |

## STIMULATOR

The electrical stimulators may be of two types, constant current stimulator and constant voltage stimulator. By varying the voltage, the constant current stimulators deliver a constant current, whereas the constant voltage stimulators alter the current in order to deliver a constant voltage. Voltage of stimulator ranges from 0-400 V and current for stimulator ranges from 0-100 mA.

The stimulus isolation unit delivers stimulus for nerve conduction study. It consists of two inductors. Magnetic field produced by an electrical stimulus

delivered to the primary coil induces a current flow in the secondary coil. This induced current is delivered to the patient. The primary and secondary coils do not have any physical connection with to the recording components of the apparatus. This reduces the shock artefact and provides protection to the patient by guarding against leakage current. Normally, depolarisation occurs beneath the cathode and hyperpolarisation occurs beneath the anode. While placing the stimulator, the cathode is positioned in such a way that it lies near the active recording electrode. If anode is placed close to active recording electrode, it may result in prolongation of latency and reduction in nerve conduction velocity values.

**STIMULUS:**

A stimulus is defined as an external agent which is sufficient to elicit a response in a cell. Electrical stimulus, used in nerve conduction studies, is defined by a waveform, duration in milliseconds and an intensity measured in Voltage or Current (milliampere). The stimulus is graded as subthreshold, threshold, submaximal, maximal, or supra maximal. Stimulus which is sufficient to produce a noticeable response is a threshold stimulus. Stimuli smaller than the threshold stimulus is subthreshold stimuli. The stimulus intensity beyond which increase in the stimulus intensity does not produce any increase in response is maximal stimuli. Stimulus intensity between threshold stimuli and maximal stimuli are submaximal stimuli. Stimulus intensity more than the maximal stimulus are called supra maximal stimuli. Supra maximal stimuli are commonly

used in nerve conduction studies. Supra maximal stimulation is given by an electric stimulus of around 20% voltage or current more than the maximal stimulus.

**Superficial peroneal nerve**:

Otherwise known as musculocutaneous nerve is a branch of common peroneal nerve. It is a mixed nerve. Supplies peroneus longus, peroneus brevis and it also supplies the cutaneous aspect of dorsum of foot, $1^{st}$ to $4^{th}$ toes dorsally.

**PROCEDURE OF NERVE CONDUCTION STUDY**

The nerve conduction study was performed in a warm room. Superficial peroneal nerve was tested on both sides. The patient was positioned in a comfortable and relaxed supine position. The skin was cleaned with spirit and the electrodes were placed using electrode jelly. Surface disc electrodes were used.

**General precautions for performing nerve conduction studies:**

- The site for placement of electrode must be cleaned and oil, grease, and soil has to be removed to reduce impedance at the 'electrode/skin interface'.
- All the electrodes are cleaned using warm soapy solution after each use
- A thin film of electrode gel is applied over each electrode to maximize conductivity

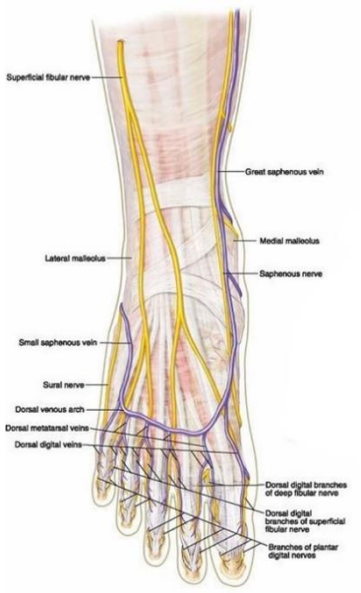




**Fig.23. Superficial peroneal nerve- Course and distribution**

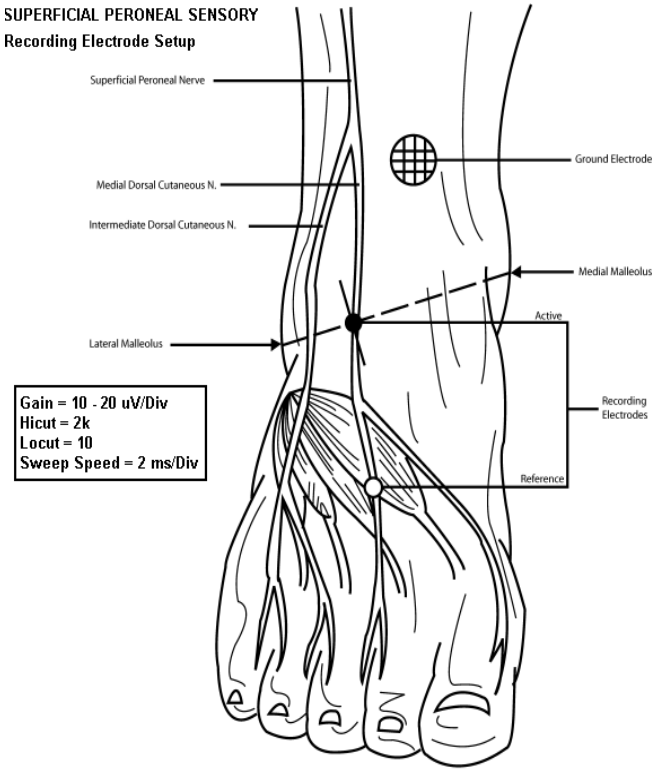
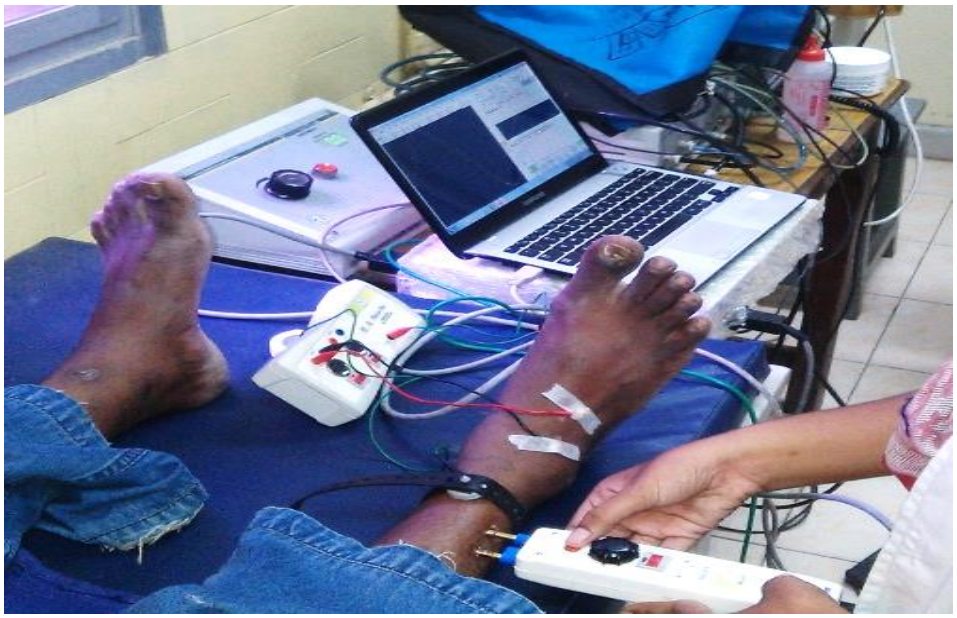


**Fig.24. Electrode placement for nerve conduction in superficial peroneal nerve**

**Photograph.3.** Nerve conduction study in Superficial Peroneal Nerve

**Photograph.4.** Electrode placement for nerve conduction study in Superficial Peroneal Nerve



- A tape must be used for clearly locating the recording and stimulating points in close apposition to anatomic course of the nerve
- The cathode (-) of the stimulating electrode must be positioned toward the active (recording) electrode for all studies

**Electrode Placement:**

**Active (Recording) Electrode:** Placed just above the lateral one-third of line connecting both malleoli.

**Reference Electrode:** Placed 3 cm distal to and in line with the active electrode.

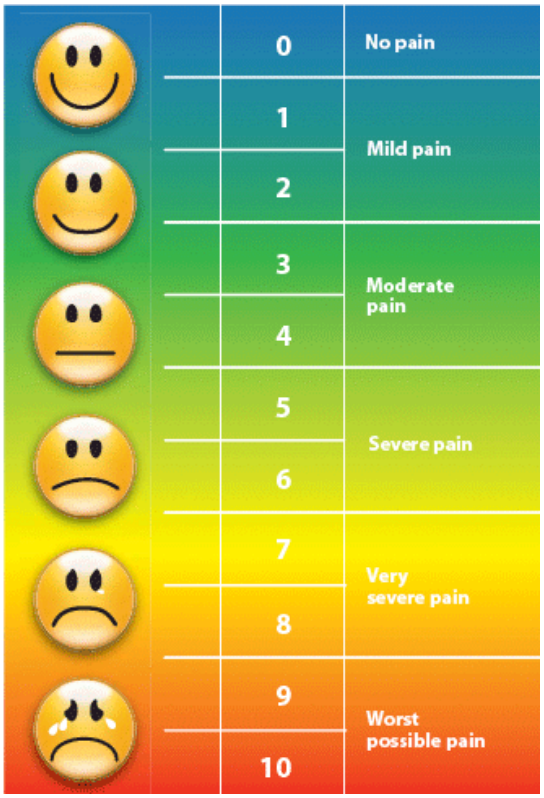
**Ground Electrode:** Placed on the crest of anterior tibia between the active and stimulating electrodes.

**Electrostimulation:** Antidromic surface stimulation was performed between 10 cm-14 cm proximal to the active electrode, just anterior to the peroneus longus tendon, along the anterolateral aspect of the leg.

**VISUAL ANALOG SCALE:**

Visual Analogue Scale (VAS) is an instrument which measures a feature such as pain that could vary across a range of values and not be directly measured (D. Gould et al., 2001[188]). For example, the extent of pain that a patient feels ranges from no pain to extreme pain. VAS is widely employed owing to its simplicity and adaptability to a wide range of populations. Pain was assessed before and after the PEMF therapy using the Visual Analog Scale.

**Photograph.5.** Visual Analog Scale




**Photograph.6.** Assessment of Vibration Perception Threshold (VPT)

**ASSESSMENT OF VIBRATION PERCEPTION THRESHOLD:**

Vibration Perception Threshold (VPT) was semi quantitatively assessed by using a simple device known as biothesiometer (or) neurothesiometer. Biothesiometer probe, vibrating at an amplitude, was applied perpendicular to the testing region using a steady pressure.

The sensation of vibration was initially familiarized to the subjects by placing the probe over the palmar surface of hand. Six points over the plantar aspect of foot were tested for Vibration Perception and the amplitude of the vibration was slowly raised until vibration could be detected by the patients. The amplitude at which the vibration was detected by the patient was taken as vibration perception threshold. The six points that were tested are in the following order, from proximal to distal: Great toe, base of 1$^{st}$ metatarsal, base of 3$^{rd}$ metatarsal, base of 5$^{th}$ metatarsal, medial plantar arch and heel. An average of these sites was taken. Value greater than 15 Volts is suggestive of an abnormal vibration perception threshold and greater VPT values strongly predict increased risk of foot ulceration (Young MJ et al., 1994[189], Armstrong DG et al., 1998[190]).

**Tc-99 MDP 3 PHASE BONE SCAN:**

Tc-99 MDP 3 phase bone scan was performed before and after PEMF at ***Advanced Nuclear Medicine Research Institute, Purasaiwalkam, Chennai.***

Tc-99 MDP acts by binding onto the surface of bone by a process called chemisorption. Strength of chemisorption is midway between chemical covalent bonding (Chemical bonding) and hydrogen bonding (adsorption).

**Photograph.7.** Vibration Perception Threshold (VPT) report

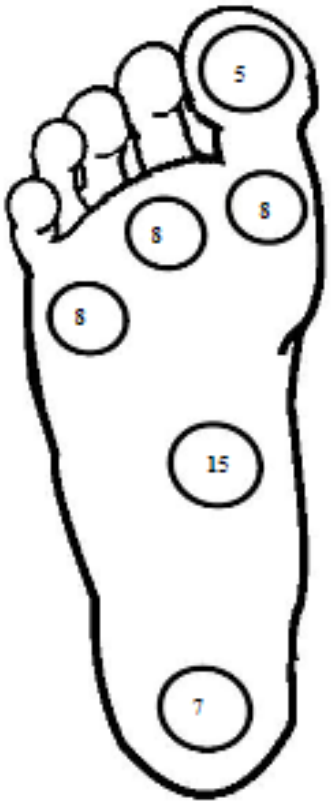
# INSTITUTE OF DIABETOLOGY

Madras Medical college&Govt.General Hospital
Chennai-600003

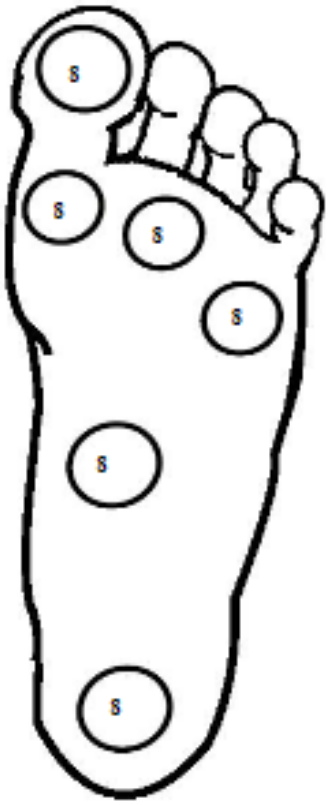
| | | | |
|---|---|---|---|
| ID | : 011020140900 | Gender | : MALE |
| Name | : Mr.paskirisamy | Date | : 01-Oct-2014 |
| Age | : 55 Yrs | Referral | : Dr. |

## BIOTHESIOMETRY STUDY

RIGHT




LEFT




AVERAGE : 8 Normal Study**
(in Volts)

8 Normal Study**

Beyond 25 Volts is 7 times more prone to ulceration in a diabetic foot than a normal foot. It is increasing to 23 times beyond 42 Volts. (Higher the threshold, higher the risk).

REMARKS :

NR - Not Recorded

CONSULTANT : Dr.Prof.P.Dharmarajan
SPECIALISATION : Diabetologist

(EXAMINER)

**Preparation:**

$^{99m}$Tc-MDP (Technicium-99 methylene diphosphonate) was synthesized at room temperature. A sterile non-pyrogenic preparation of sodium pertechnetate ($^{99m}$Tc) was injected into a vial containing the lyophilized mixture of medronic acid, stannous chloride dehydrate and p-aminobenzoic acid. Contents of the vial was dissolved by swirling gently. Recommended dose of Tc 99m MDP is 20 to 25 millicurie (mCi) (750 to 900 megabecquerel [MBQ])

**Procedure:**

Radiopharmaceutical is injected in to the cubital vein of the patient. Individual is made to lie facing up on a table during the procedure. The gamma camera, a scanning device is positioned over the region of interest. It then moves over the body detecting and recording regions of active and inactive metabolism. The images thus obtained are then viewed using a special computer.

Three-phase bone scan consists of:

**Phase 1:**

- Nuclear angiogram or the flow phase
- Dynamic imaging
- After the intravenous injection of the radiopharmaceutical, images are obtained at 1 second interval for 60 seconds
- Reflects vascularity

**Phase 2:**

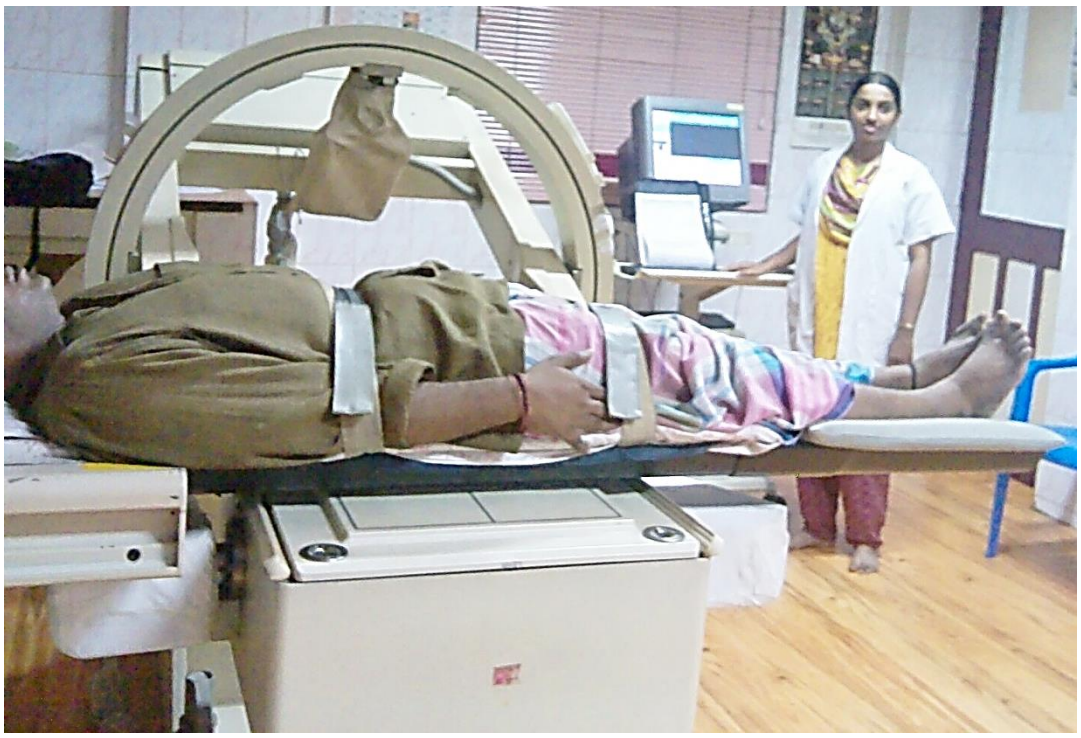
- Blood pool phase
- Images are acquired 5 minutes, 1 hour and 3 hours after injection.
- Static imaging
- Reflects the level of soft tissue uptake of radioactive material
- Stagnant blood flow due to dilated capillaries, usually seen in areas of moderate and severe inflammation, are seen as radioisotope pooling in this phase

**Phase 3:**

- Delayed phase
- Static imaging
- Traditional bone scan
- Images are acquired 2 to 4 hours after injection, when most of the radioisotope has been metabolized.

| **Phase** | **Name** | **Time after Injection** | **Purpose** |
|---|---|---|---|
| One | Radionuclide Angiogram | Immediate | Reflects vascularity |
| Two | Blood pool | Few minutes, 1 hour, 3 hour | Reflects soft tissue Involvement |
| Three | Delayed | 2 to 4 hours | Reflects osteoblastic Response |

**Photograph.8.** Tc-99 MDP bone scan for a patient at Advanced Nuclear Medicine Research Institute, Chennai



**Photograph.9.** Blood samples for estimation of Erythrocyte Superoxide Dismutase (SOD)

**ESTIMATION OF ERYTHROCYTE SUPER OXIDE DISMUTASE LEVELS:**

About 2 ml of blood was collected in EDTA tubes before and after PEMF. The blood was centrifuged at 1000 x g for 10 minutes at 4$^{o}$C. The plasma along with white buffy coat was pipetted out. The Red Blood Cells were lysed with double distilled water (4 times the volume of RBCs) and centrifuged at 10,000 x g for 15 minutes at 4$^{o}$C and the supernatant was collected and stored in deep freezer at –80$^{o}$C until estimating superoxide dismutase levels. The samples were transported in ice to Institute of Basic Medical Sciences, Taramani and assayed as follows.

**Principle (**Marklund and Marklund[212], 1974)

Pyrogallol auto oxidizes rapidly in an aqueous solution at a faster rate with higher pH (8.0) to produce various intermediate products. The inhibition of pyrogallol auto oxidation by the enzyme present in the sample is employed in the quantification of activity of SOD. The inhibition of auto oxidation brought about by the addition of enzyme is evaluated at the early stage as an increase in absorbance at 420 nm.

**Reagents**

1. 'Tris-HCl Buffer (0.1M; pH-8.2)': 1.576 gm of Tris-HCl was dissolved in distilled water and the pH was adjusted to 8.2 using 1N NaOH and made up to 100 ml with distilled water.
2. Pyrogallol (0.2mM): 126 mg of pyrogallol was dissolved in 100 ml of Tris-HCl buffer. This solution was prepared just before use.

3. Ethylene diamine tetra acetic acid (EDTA-1mM): 29.22 mg of EDTA was dissolved in 100 ml of distilled water.
4. Diethylene triamine pentaacetic acid (DTPA-1mM): 19.67 mg of DTPA was dissolved in 50 ml of distilled water.

**Procedure:**

A mixture containing 2.5 ml of Tris-HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA was prepared. To this mixture, 0.5 ml of pyrogallol was added and the increase in absorbance was read at 420 nm against the blank for 3 minutes to determine the rate of auto oxidation of pyrogallol. 100 µl of tissue homogenate was mixed with 2.5 ml of Tris-HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA. To this mixture, 0.5 ml of pyrogallol was added and the increase in absorbance was read at 420 nm using a spectrophotometer against the blank for a period of 3 minutes. This measurement constituted the rate of inhibition of auto oxidation of pyrogallol brought about by the enzyme present in the tissue homogenate. The reagent blank contained a mixture of 3.1 ml of Tris- HCl buffer, 0.1 ml of EDTA and 0.5 ml of DTPA and this was used to set 100% absorbance. The activity of SOD in tissues is expressed as Units/g of hemoglobin.

## STATISTICAL ANALYSIS

Statistical analysis was done using the software SPSS version 21.

**Paired Student's t test** was carried out to compare the means of variables before and after administration of Pulsed Electro Magnetic Field therapy.

# RESULTS

# RESULTS

## 5.1. CHARACTERISTICS OF THE STUDY POPULATION

| TABLE 1 | | |
|---|---|---|
| **BASELINE PARAMETERS** | | |
| **S. No** | **Variable** | **Mean ± SD** |
| 1. | Age (in years) | 57.1 ± 5.72 |
| 2. | Duration of diabetes (in years) | 13.87 ± 4.19 |
| 3. | Duration of diabetic neuropathy symptoms (in years) | 2.23 ± 1.63 |
| 4. | Dyck's Neuropathic Symptom Score | 2.83 ± 0.79 |
| 5. | Blood sugar level (mg/dl) | 114.77 ± 16.92 |
| 6. | Blood urea (mg/dl) | 23.07 ± 2.41 |
| 7. | Serum creatinine (mg/dl) | 0.85 ± 0.17 |
| 8. | Haemoglobin (g/dl) | 11.72 ± 1.23 |
| 9. | Peripheral smear | Normal study |
| 10. | Total Cholesterol (mg/dl) | 156.27 ± 19.85 |
| 11. | Electrocardiogram | Normal |
| 12. | Fundus examination | Normal |

The mean age of the individuals included in the present study was 57.1 ± 5.72 years with the range of 40 to 60 years. With regard to the gender, equal number of males and females participated in the study. i.e. 15 males and 15 females. The mean duration of diabetes mellitus in study subjects was 13.87± 4.19 years and the mean duration of diabetic neuropathy symptoms was 2.23 ±

1.63. The average Dyck's Neuropathic Symptom Score (DNSS) was found to be 2.83 ± 0.79 ranging from 1 to 4. The mean blood sugar level was 114.77 ± 16.92 mg/dl. The mean haemoglobin was found to be 11.72 ± 1.23 g/dl. The average total cholesterol of the study group was 156.27 ± 19.85. Renal function tests were normal with mean blood urea of 23.07 ± 2.41 mg/dl and mean serum creatinine levels of 0.85 ± 0.17 mg/dl. The individuals of the study group had normal picture of peripheral smear. Their fundus examination and electrocardiogram were normal indicating that they didn't have any other microvascular complications of diabetes.

### 5.2. VISUAL ANALOG SCORE (VAS):

The mean values of Visual Analog Score before and after PEMF are represented in table 2 and graph 1. The Visual Analog Score is shown in photograph 5.

**TABLE 2**

Comparison of Visual Analog Score before and after Pulsed Electro Magnetic Field therapy

| Variable | Group | N | Mean | SD | P –Value |
|---|---|---|---|---|---|
| Visual Analog Score | Before PEMF | 30 | 9.5 | 0.67 | 0.000** |
| | After PEMF | 30 | 0.63 | 0.91 | |

** P – Value < 0.001 Very Highly Significant

The mean Visual Analog Score (VAS) was found to be significantly reduced (p <0.001) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

### 5.3. VIBRATION PERCEPTION THRESHOLD (VPT):

The mean values of Vibration Perception Threshold (VPT) before and after PEMF are represented in table 3 and graph 2. Assessment and report of the Vibration Perception Threshold are depicted in photograph 6 and 7.

**TABLE 3**

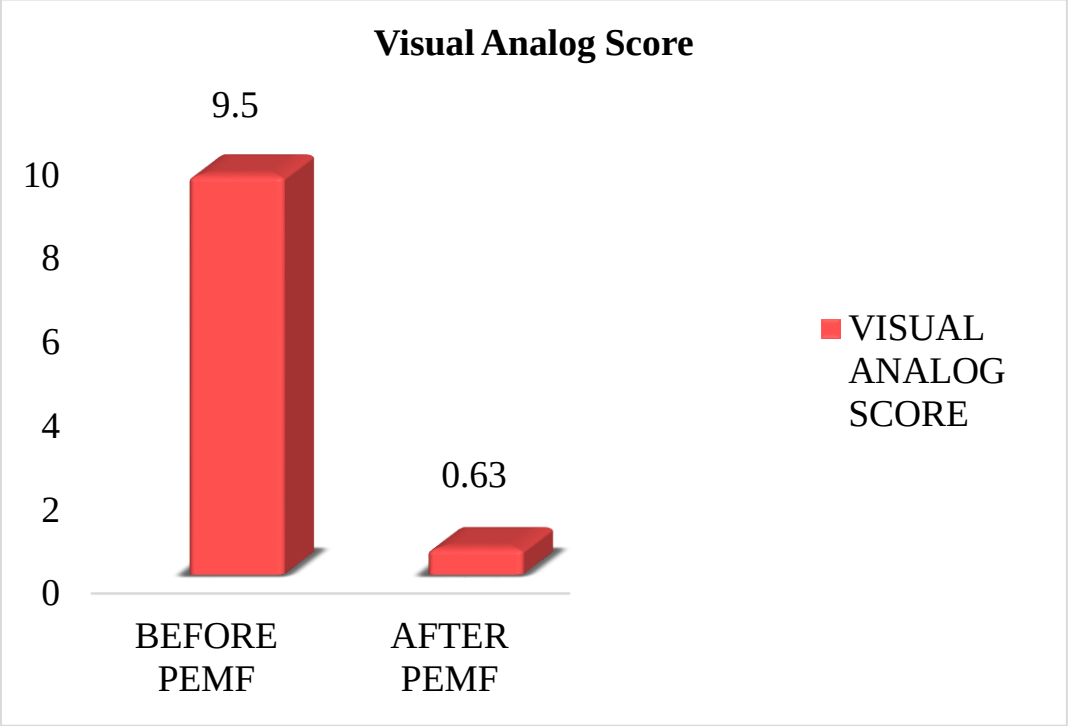
Comparison of mean values of Vibration Perception Threshold before and after Pulsed Electromagnetic Field Therapy

| Variable | Group | Site | Mean | SD | P –Value |
|---|---|---|---|---|---|
| Vibration Perception Threshold (Volts) | Before PEMF | Right foot | 34.91 | 10.14 | 0.000** |
| | After PEMF | | 16.81 | 8.04 | |
| | Before PEMF | Left foot | 34.10 | 10.20 | 0.000** |
| | After PEMF | | 16.18 | 7.26 | |

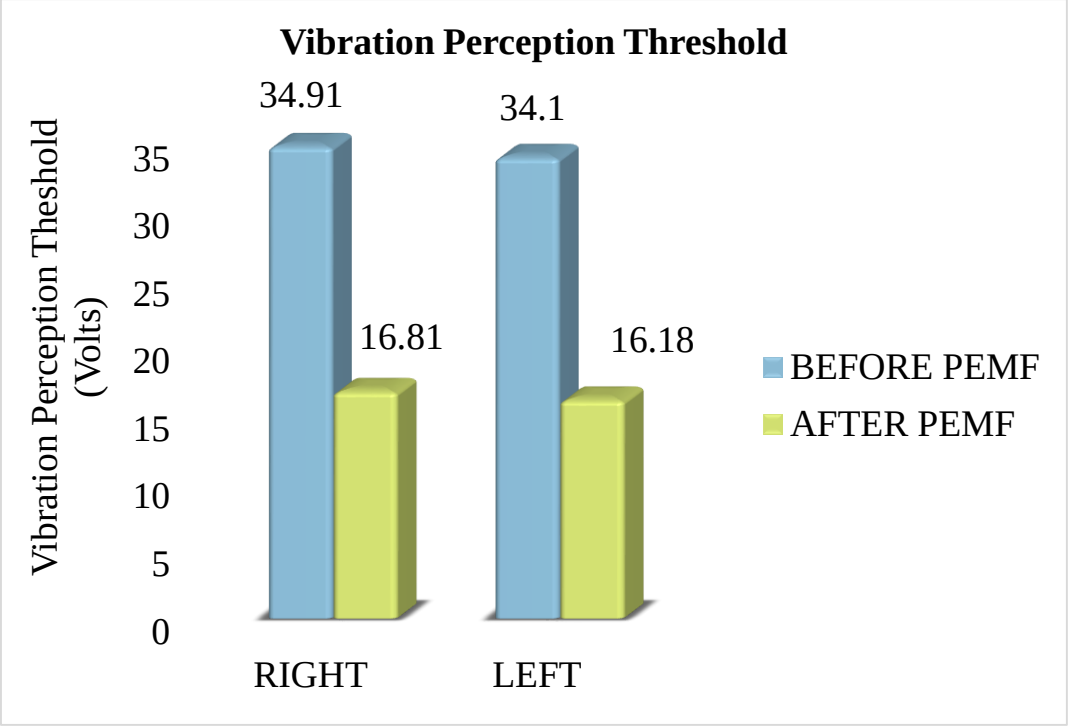
** P – Value < 0.001 Very Highly Significant

The mean Vibration Perception Threshold (VPT) in both foot was found to be significantly reduced (p <0.001) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

**Graph.1.** Comparison of Visual Analog Score (VAS) before and after PEMF therapy




**Graph.2.** Comparison of mean values of Vibration Perception Threshold (VPT) before and after PEMF therapy



## 5.4. NERVE CONDUCTION PARAMETERS:

Variables pertaining to nerve conduction study in Superficial Peroneal Nerve in the study group before and after Pulsed Electro Magnetic Field therapy are furnished in the tables 4, 5 and 6. They are also represented in the graphs 3, 4 and 5. The displayed images of the waveforms recorded using the computerised nerve conduction testing equipment before and after PEMF therapy have been given as Photograph 10.

**TABLE 4**

Comparison of mean values of latency of Sensory Nerve Action Potential (SNAP) in Superficial Peroneal Nerve before and after PEMF therapy

| Variable | Group | Side | Mean | SD | P –Value |
|---|---|---|---|---|---|
| Latency of SNAP in Superficial Peroneal Nerve (in milliseconds) | Before PEMF | Right | 4.28 | 0.62 | 0.000* |
| | After PEMF | | 2.80 | 0.51 | |
| | Before PEMF | Left | 4.25 | 0.63 | 0.000** |
| | After PEMF | | 2.53 | 0.60 | |

* P – Value < 0.001 Very Highly Significant

The mean latency of SNAP in Superficial Peroneal Nerve was found to be significantly reduced ($p < 0.001$) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

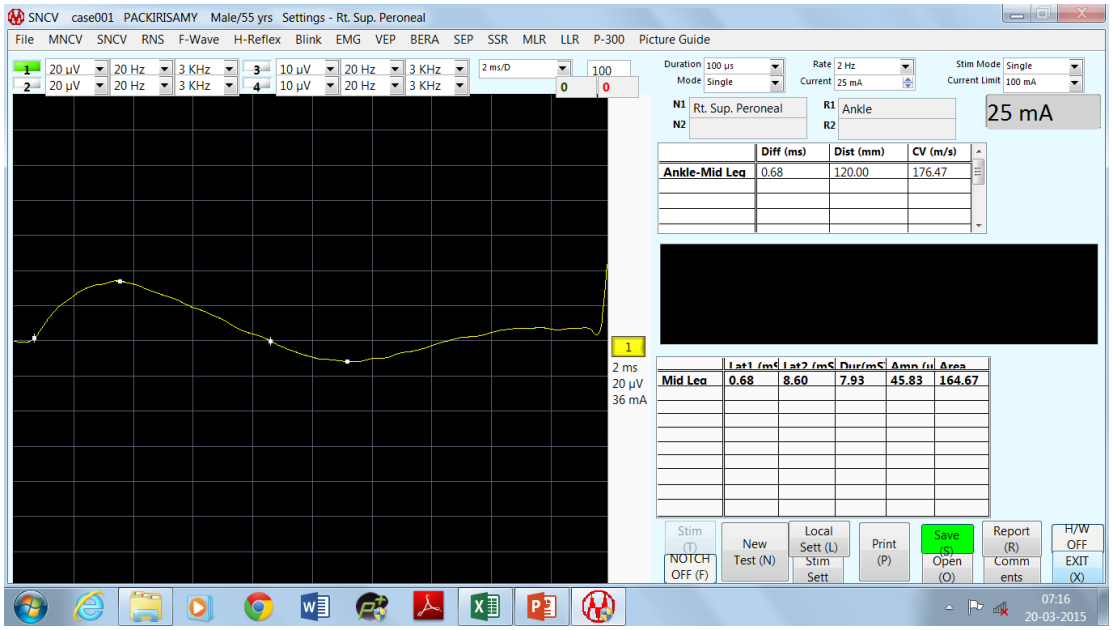
| TABLE 5 | | | | | |
|---|---|---|---|---|---|
| Comparison of mean values of amplitude of Sensory Nerve Action Potential (SNAP) in Superficial Peroneal Nerve before and after PEMF therapy | | | | | |
| Variable | Group | Side | Mean | SD | P –Value |
| Amplitude of SNAP in Superficial Peroneal nerve (in µVolts) | Before PEMF | Right | 11.12 | 0.94 | 0.000** |
| | After PEMF | | 17.18 | 0.75 | |
| | Before PEMF | Left | 11.32 | 1.03 | 0.000** |
| | After PEMF | | 17.51 | 0.89 | |
| ** P – Value < 0.001 Very Highly Significant | | | | | |

The mean amplitude of SNAP in Superficial Peroneal Nerve was found to be significantly increased (p <0.001) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

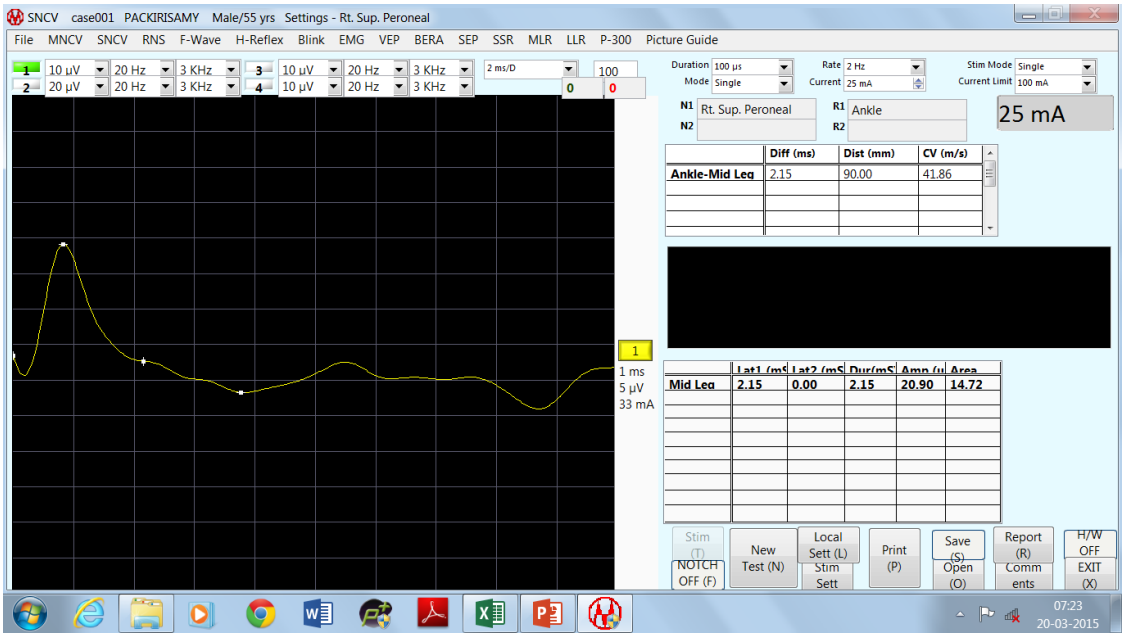
| TABLE 6 | | | | | |
|---|---|---|---|---|---|
| Comparison of mean values of Sensory Nerve Conduction Velocity (NCV) of Superficial Peroneal Nerve before and after PEMF therapy | | | | | |
| Variable | Group | Side | Mean | SD | P -Value |
| Sensory Nerve Conduction Velocity (NCV) of Superficial Peroneal Nerve (metre/seconds) | Before PEMF | Right | 28.63 | 4.41 | 0.000** |
| | After PEMF | | 44.59 | 9.65 | |
| | Before PEMF | Left | 28.82 | 4.28 | 0.000** |
| | After PEMF | | 49.49 | 9.64 | |
| ** P – Value < 0.001 Very Highly Significant | | | | | |

**Photograph.10.** Sensory Nerve Action Potential Recording before and after PEMF

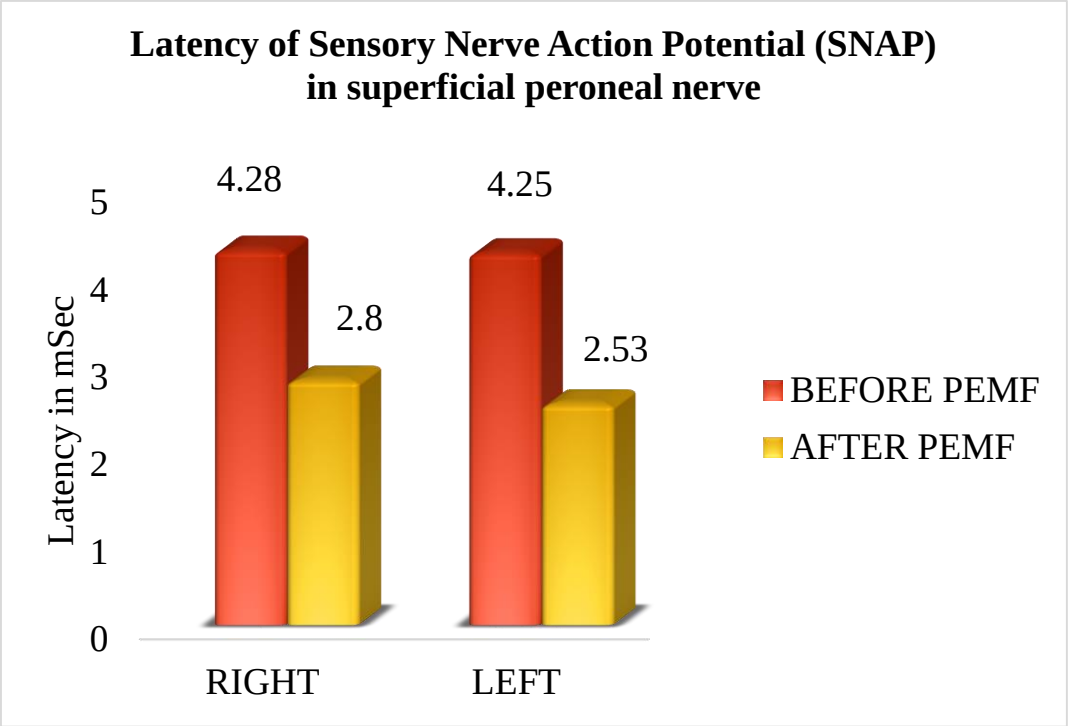
Sensory Nerve Action Potential Recording before PEMF



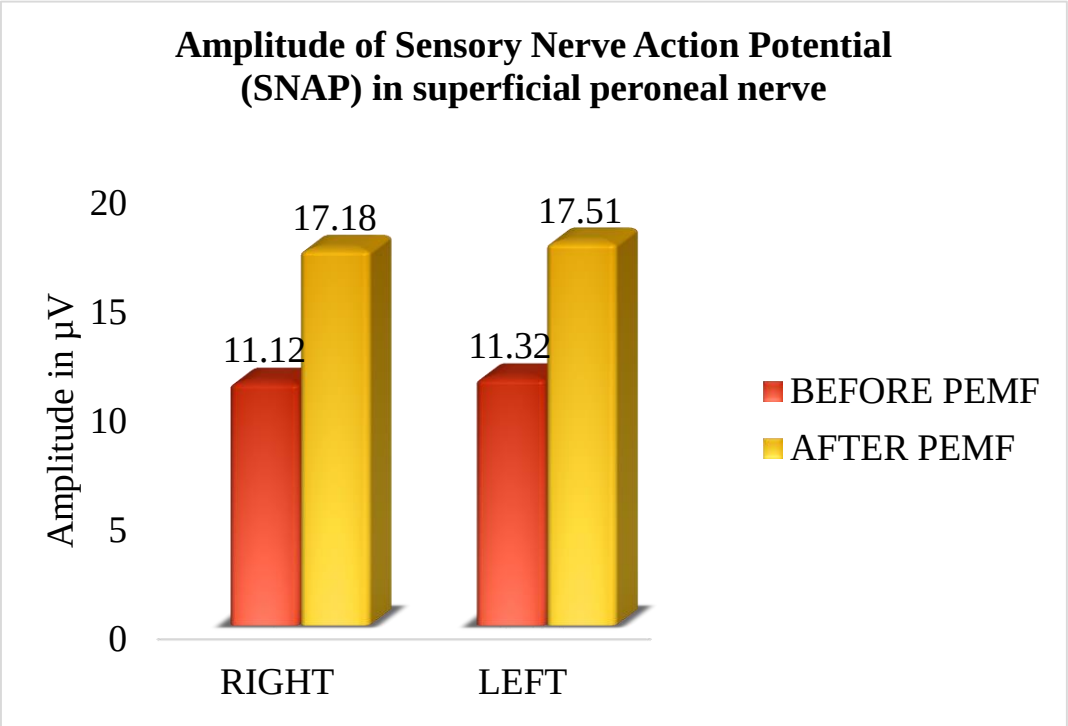

Sensory Nerve Action Potential Recording after PEMF



**Graph.3.** Comparison of mean values of latency of Sensory Nerve Action Potential (SNAP) in Superficial Peroneal Nerve before and after PEMF therapy




**Graph.4.** Comparison of mean values of amplitude of Sensory Nerve Action Potential (SNAP) in Superficial Peroneal Nerve before and after PEMF therapy



The mean Sensory Nerve Conduction Velocity (NCV) of Superficial Peroneal Nerve was found to be significantly increased (p <0.001) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

### 5.5. Erythrocyte Super Oxide Dismutase levels:

The mean values of Erythrocyte Superoxide dismutase levels before and after PEMF are represented in table 7 and graph 6.

**TABLE 7**

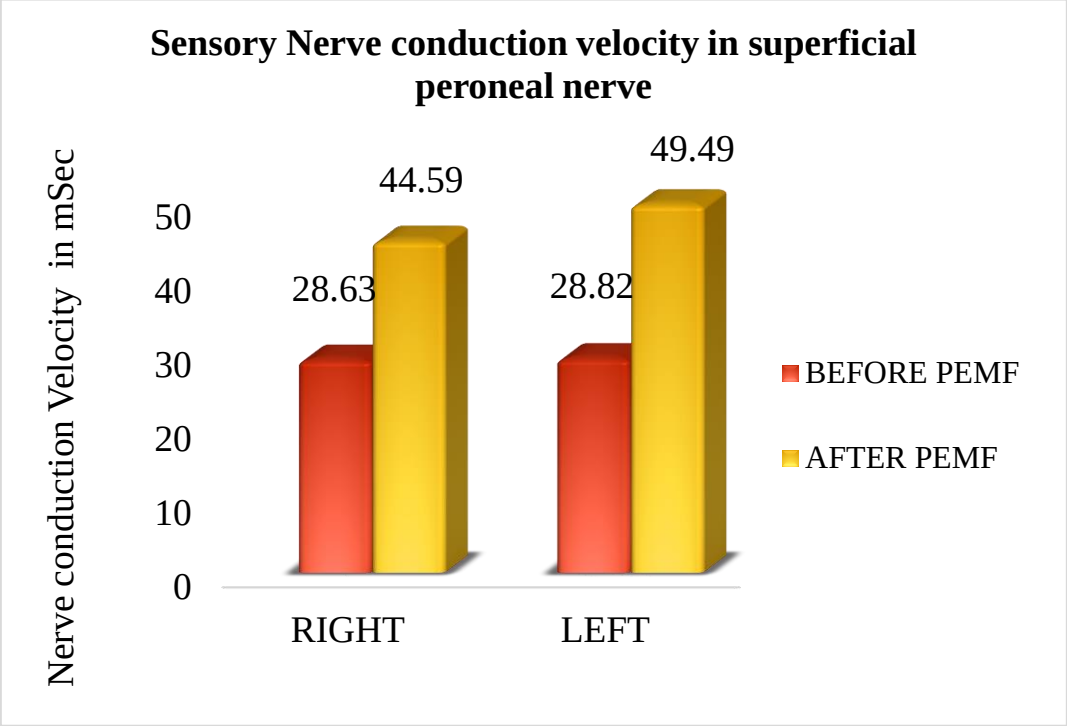
Comparison of mean values of erythrocyte Super Oxide Dismutase levels before and after PEMF therapy

| Variable | Group | N | Mean | SD | P - Value |
|---|---|---|---|---|---|
| Erythrocyte Super Oxide Dismutase levels (U/g hemoglobin) | Before PEMF | 30 | 329.73 | 10.71 | 0.000** |
| | After PEMF | 30 | 376.53 | 8.19 | |

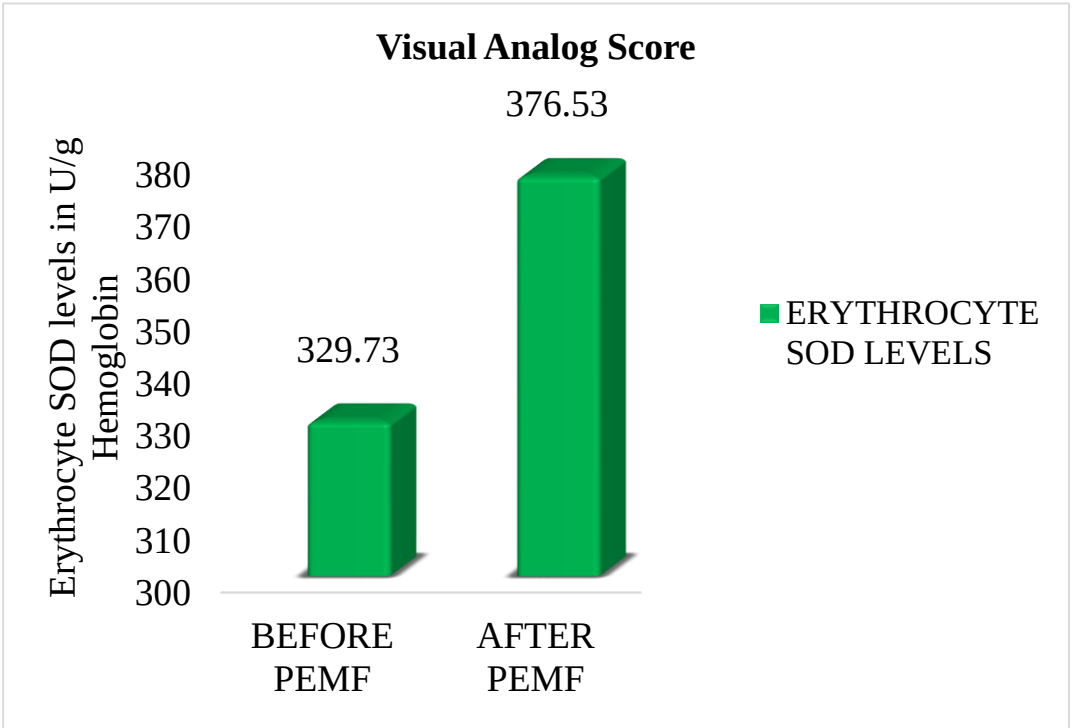
** P – Value < 0.001 Very Highly Significant

The mean erythrocyte superoxide dismutase level was found to be significantly increased (p<0.001) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

**Graph.5.** Comparison of mean values of Sensory Nerve Conduction Velocity (NCV) of Superficial Peroneal Nerve before and after PEMF therapy




**Graph.6.** Comparison of mean values of erythrocyte Super Oxide Dismutase (SOD) levels before and after PEMF therapy



### 5.6. Tc-99 MDP Bone Scan Dynamic Phase:

The mean values of integral 60-120 seconds and time to ½ max. in the dynamic phase of Tc-99 MDP bone scan before and after PEMF are represented in table 8 and graphs 7, 8. The photograph of dynamic phase of Tc-99 MDP bone scan before and after PEMF is depicted in photograph 11.

**TABLE 8**

Comparison of mean values of integral 60-120 seconds and time to ½ max. at both ankles in Tc-99 MDP 3 phase bone scan (Dynamic) before and after PEMF therapy

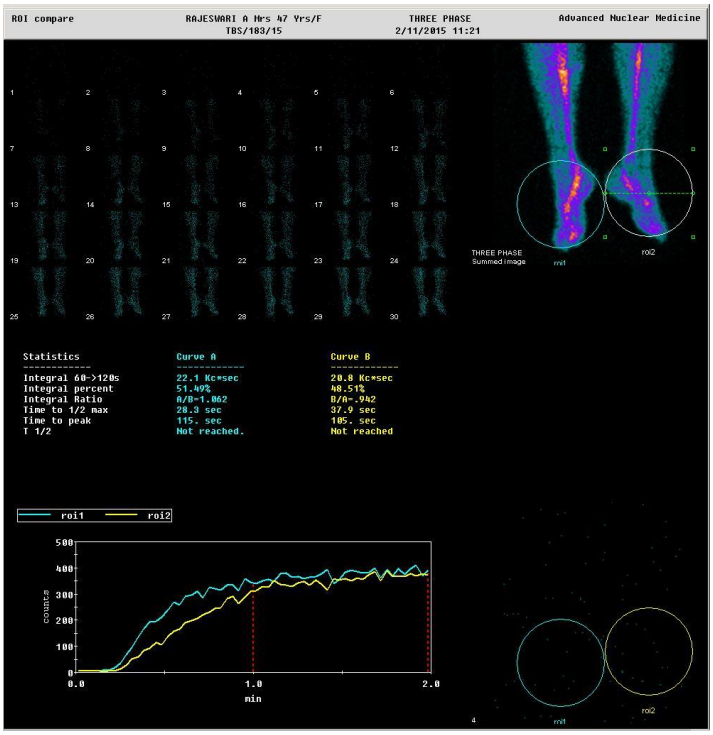
| Variable | Group | Site | Mean | SD | P -Value |
|---|---|---|---|---|---|
| Integral 60-120 seconds in Tc-99 MDP 3 phase bone scan (1000counts *seconds) | Before PEMF | Right ankle | 7.69 | 5.32 | 0.000** |
| | After PEMF | | 14.01 | 7.73 | |
| | Before PEMF | Left ankle | 7.48 | 5.68 | 0.000** |
| | After PEMF | | 13.81 | 8.68 | |
| Time to ½ max. in Tc-99 MDP 3 phase bone scan (Seconds) | Before PEMF | Right ankle | 33.80 | 13.52 | 0.000** |
| | After PEMF | | 16.46 | 6.81 | |
| | Before PEMF | Left ankle | 37.39 | 13.19 | 0.000** |
| | After PEMF | | 18.91 | 9.70 | |

** P – Value < 0.001 Very Highly Significant

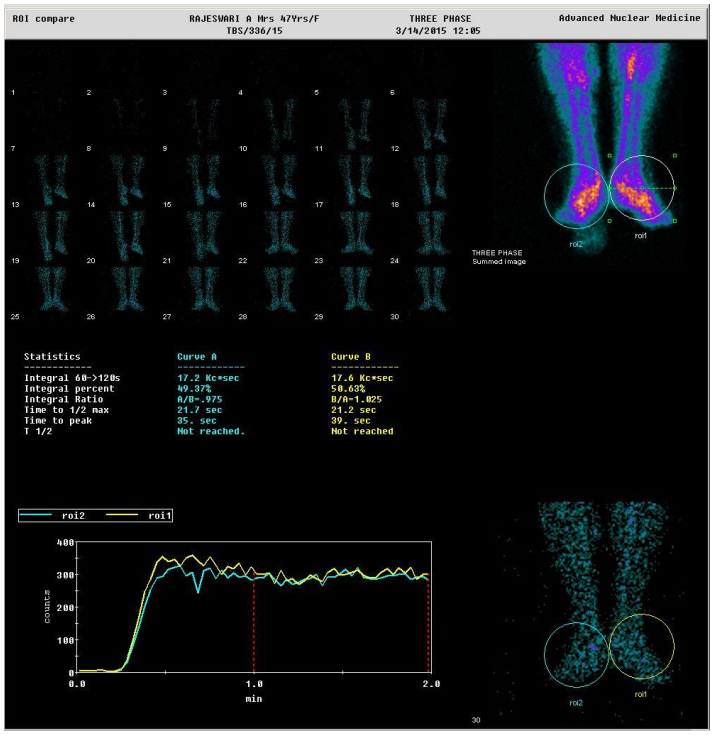
The mean integral 60-120 seconds was found to be significantly increased ($p<0.001$) and the time to ½ max. was significantly reduced ($p<0.001$) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

**Photograph.11.** Tc-99 MDP 3 phase bone scan (Dynamic phase) report before and after PEMF

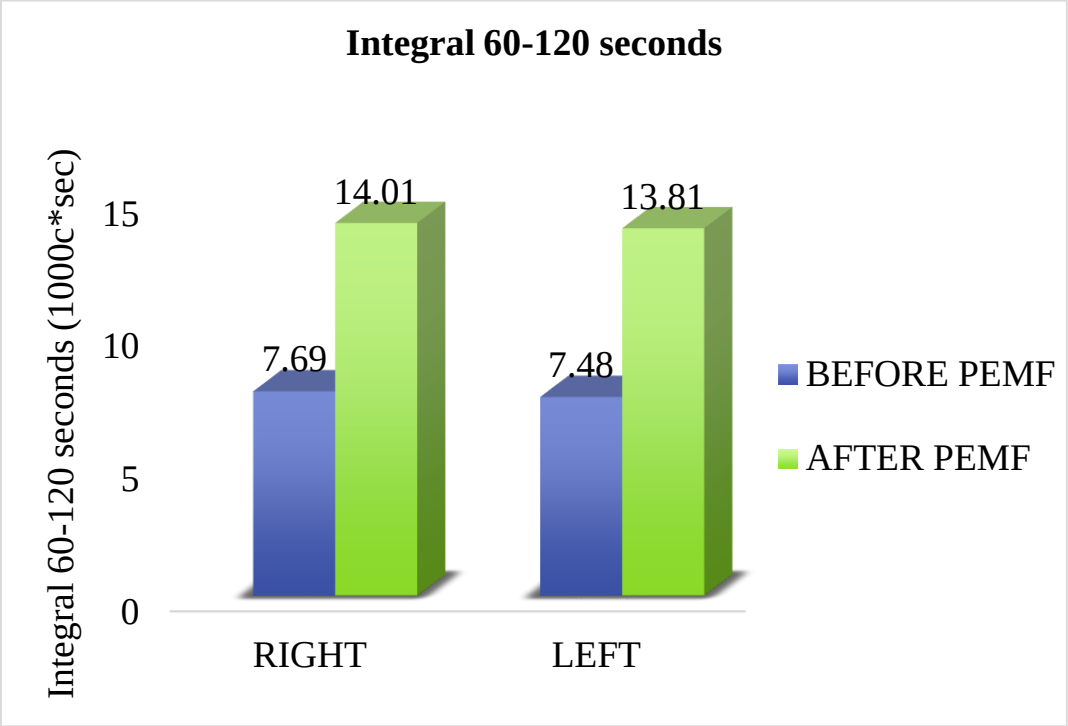
Tc-99 MDP 3 phase bone scan (Dynamic phase) report before PEMF



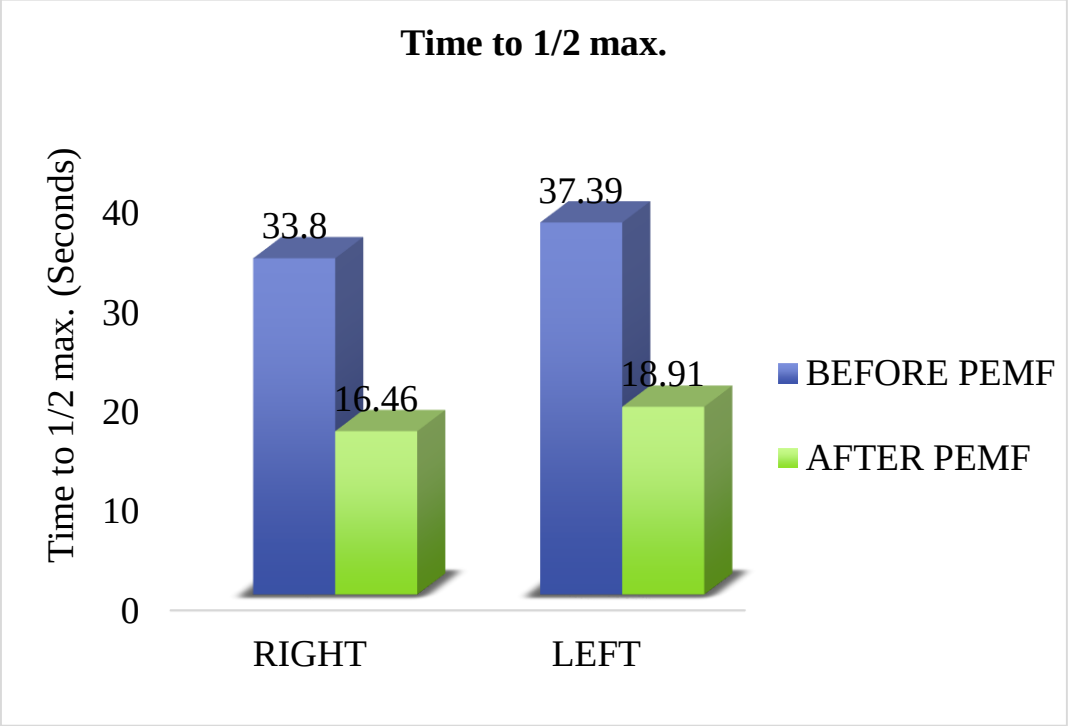

Tc-99 MDP 3 phase bone scan (Dynamic phase) report after PEMF



**Graph.7.** Comparison of mean values of integral 60-120 seconds of Tc-99 MDP 3 phase bone scan (Dynamic) before and after PEMF therapy




**Graph.8.** Comparison of mean values of time to ½ max. of Tc-99 MDP 3 phase bone scan (Dynamic) before and after PEMF therapy

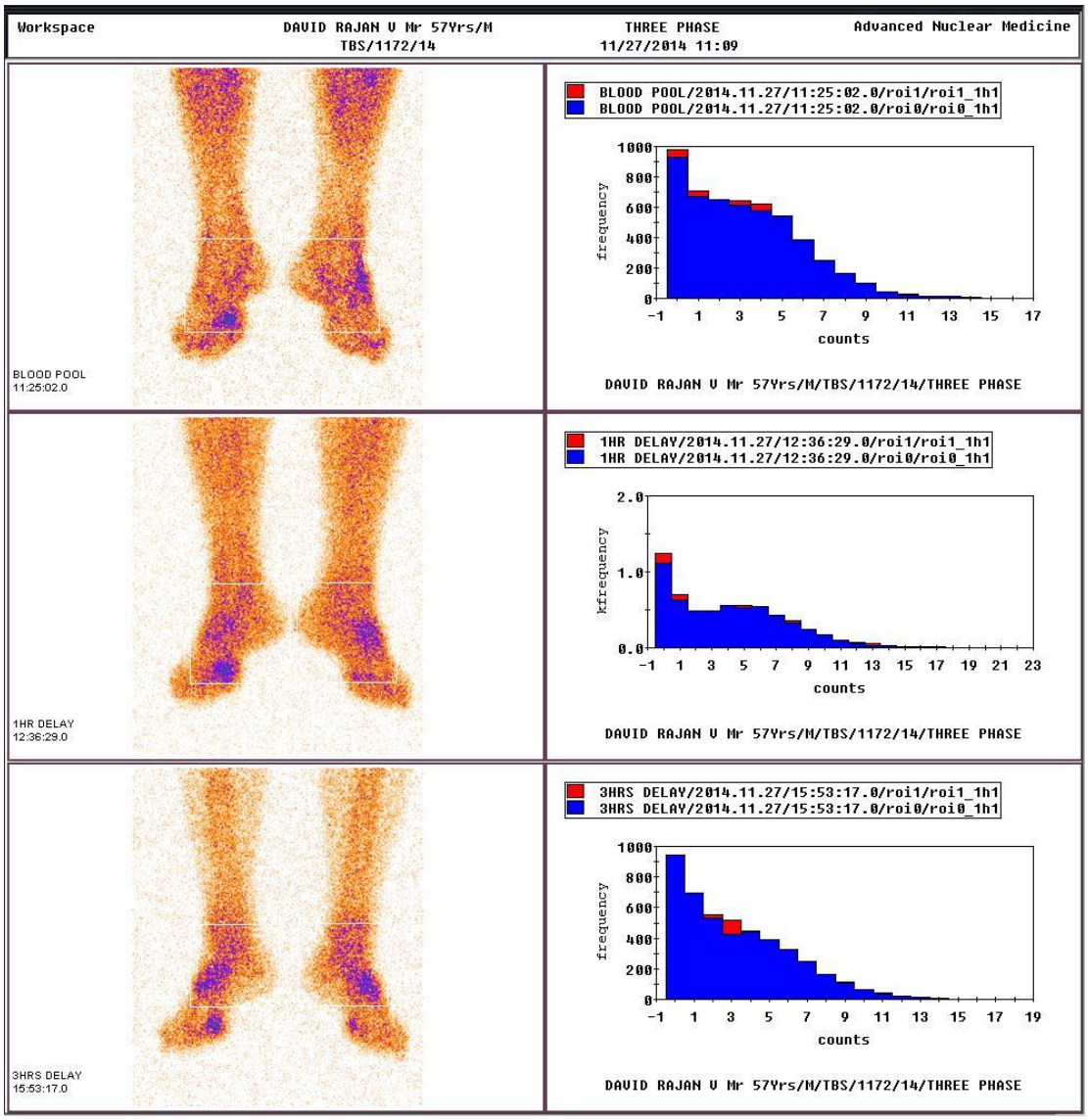


### 5.7. Tc-99 MDP Bone Scan Static phase:

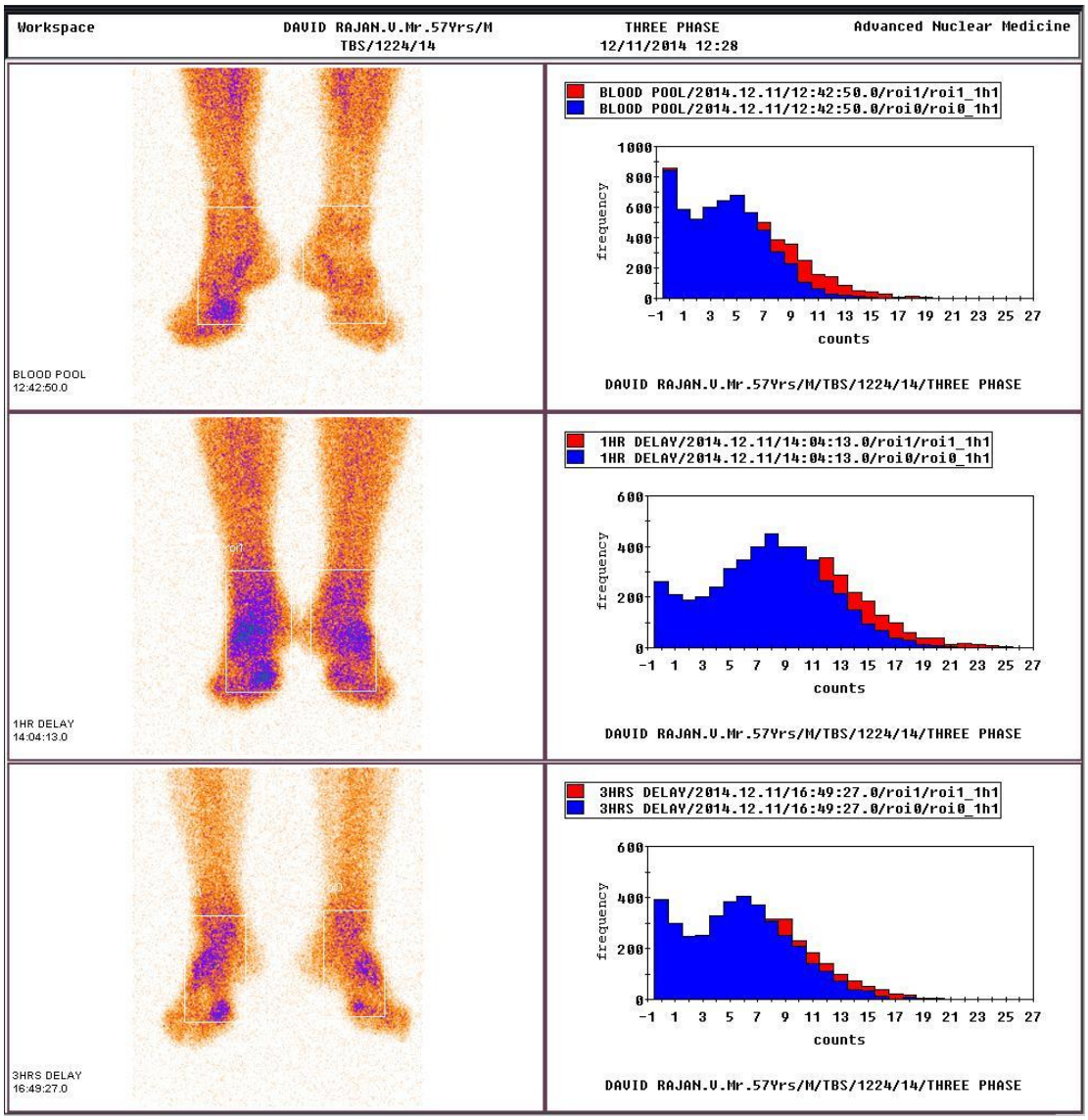
| TABLE 9 | | | | | |
|---|---|---|---|---|---|
| Comparison of mean values of blood pool, 1 hour delay and 3 hours delay frequency at both ankles in Tc-99 MDP 3 phase bone scan (Static phase) before and after PEMF therapy | | | | | |
| Variable | Group | Site | Mean | SD | P - Value |
| Blood pool frequency in Tc-99 MDP 3 phase bone scan (Hertz) | Before PEMF | Right ankle | 708.33 | 366.33 | 0.000** |
| | After PEMF | | 1194.33 | 509.45 | |
| | Before PEMF | Left ankle | 794.33 | 444.92 | 0.000** |
| | After PEMF | | 1339.33 | 612.05 | |
| One hour delay frequency in Tc-99 MDP 3 phase bone scan (Hertz) | Before PEMF | Right ankle | 651 | 324.05 | 0.000** |
| | After PEMF | | 1189 | 549.46 | |
| | Before PEMF | Left ankle | 734.20 | 379.92 | 0.000** |
| | After PEMF | | 1337.33 | 592.20 | |
| Three hour delay frequency in Tc-99 MDP 3 phase bone scan (Hertz) | Before PEMF | Right | 740.33 | 448.41 | 0.000** |
| | After PEMF | | 1297.67 | 646.21 | |
| | Before PEMF | Left | 886 | 549.08 | 0.000** |
| | After PEMF | | 1343.33 | 637.28 | |
| ** P – Value < 0.001 Very Highly Significant | | | | | |

The mean frequency in blood pool after 5 minutes, 1 hour delay and 3 hours delay showed a significant increase ($p<0.001$) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

**Photograph.12.** Tc-99 MDP 3 phase bone scan (Static phase) report before PEMF

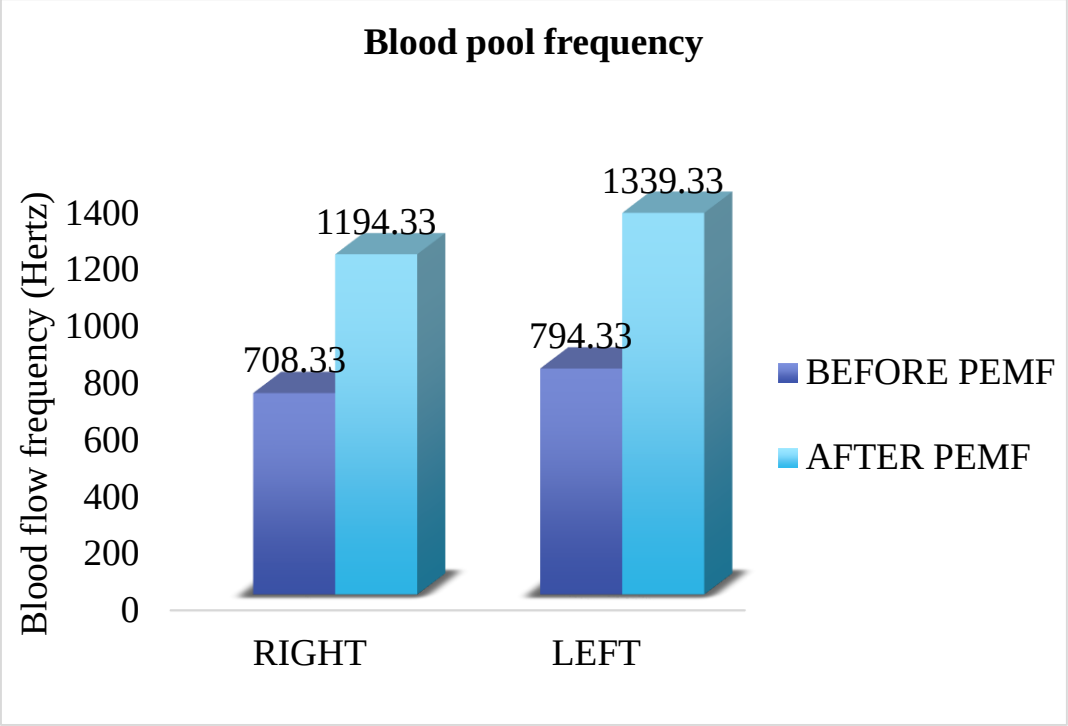


**Photograph.13.** Tc-99 MDP 3 phase bone scan (Static phase) report after

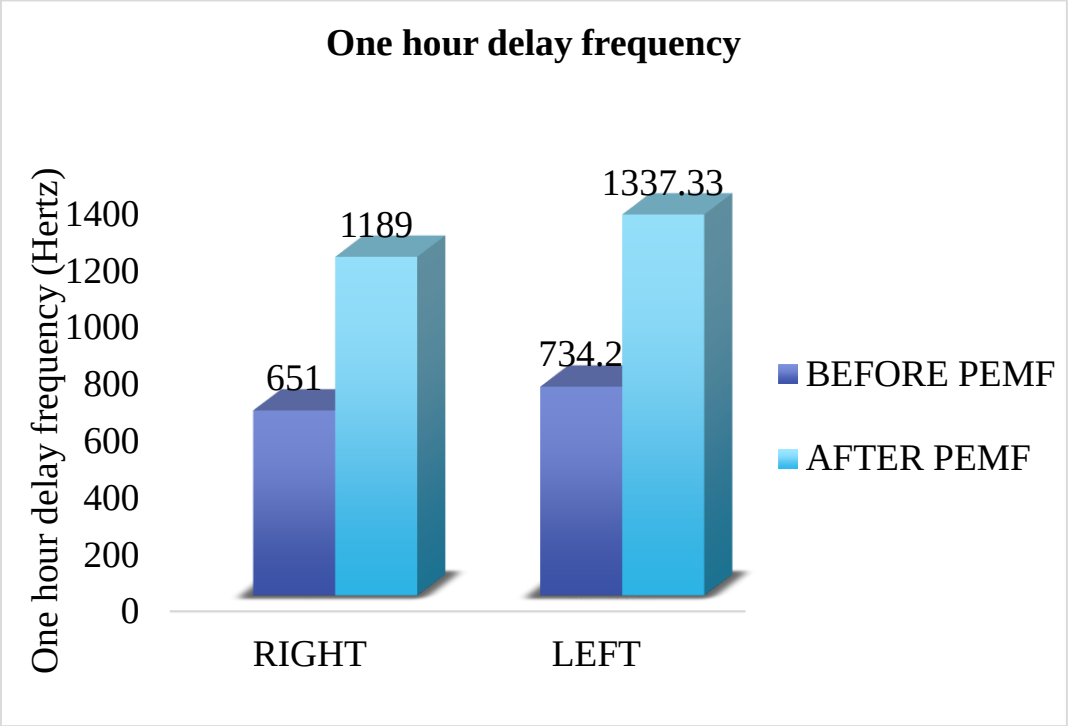



PEMF

**Graph.9.** Comparison of blood pool frequency of Tc-99 MDP 3 phase bone scan (Static) before and after PEMF therapy




**Graph.10.** Comparison of one hour delay frequency of Tc-99 MDP 3 phase bone scan (Static) before and after PEMF therapy



### 5.8. Tc-99 MDP Bone Scan delayed phase:

The mean values of bone uptake of Tc-99 MDP bone scan before and after PEMF are represented in table 10 and graphs 11, 12. The photograph of bone scan delayed phase of Tc-99 MDP bone scan before and after PEMF is depicted in photograph 14 and 15.

**TABLE 10**

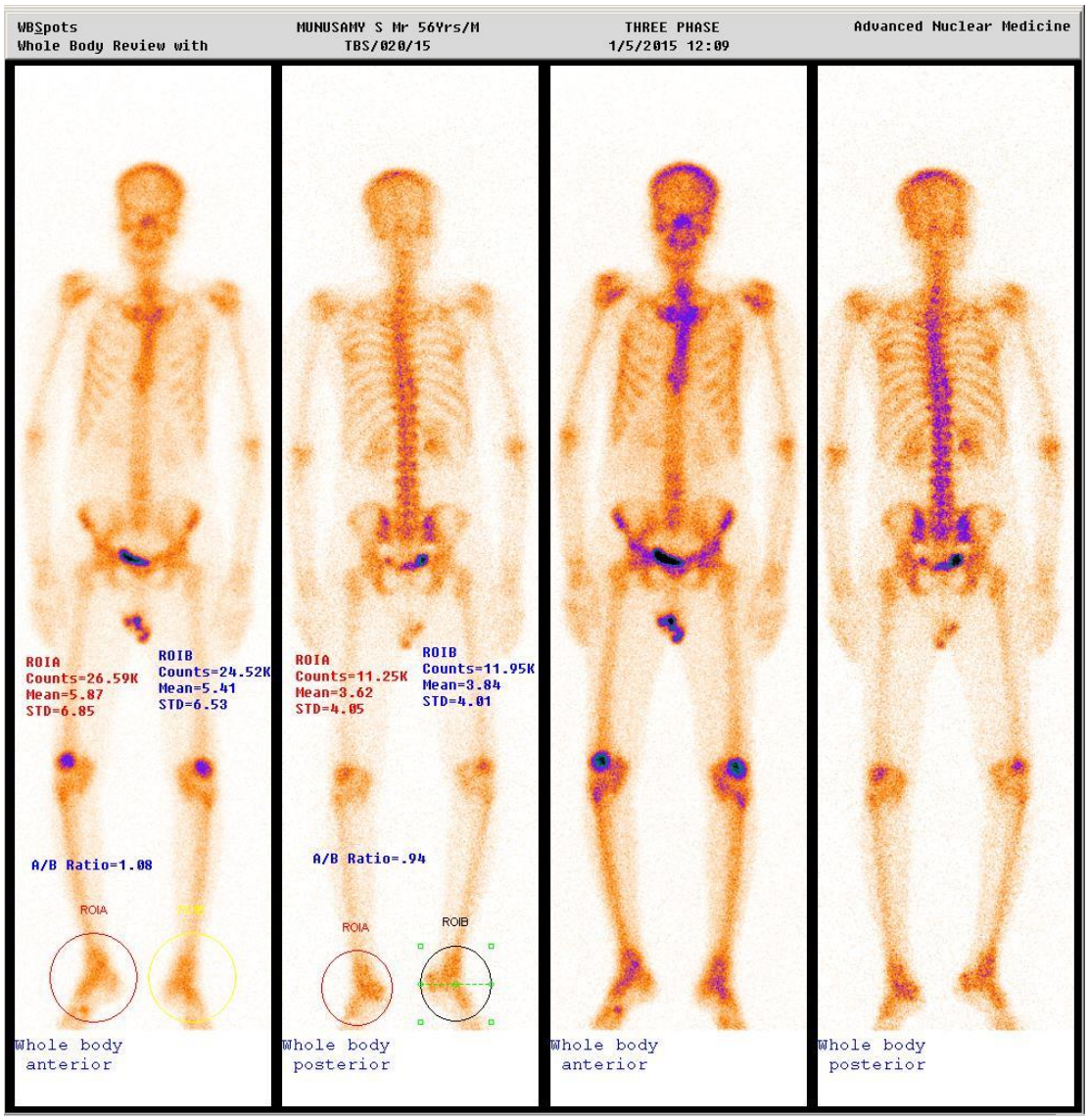
Comparison of mean values of bone uptake of Tc-99 MDP over both ankles before and after PEMF therapy

| Variable | Group | Side | Mean | SD | P -Value |
|---|---|---|---|---|---|
| Bone scan counts before and after PEMF (X 1000) | Before PEMF | Right ankle | 40.34 | 16.62 | 0.001** |
| | After PEMF | | 57.11 | 30.80 | |
| | Before PEMF | Left ankle | 40.87 | 18.80 | 0.000** |
| | After PEMF | | 56.94 | 29.27 | |

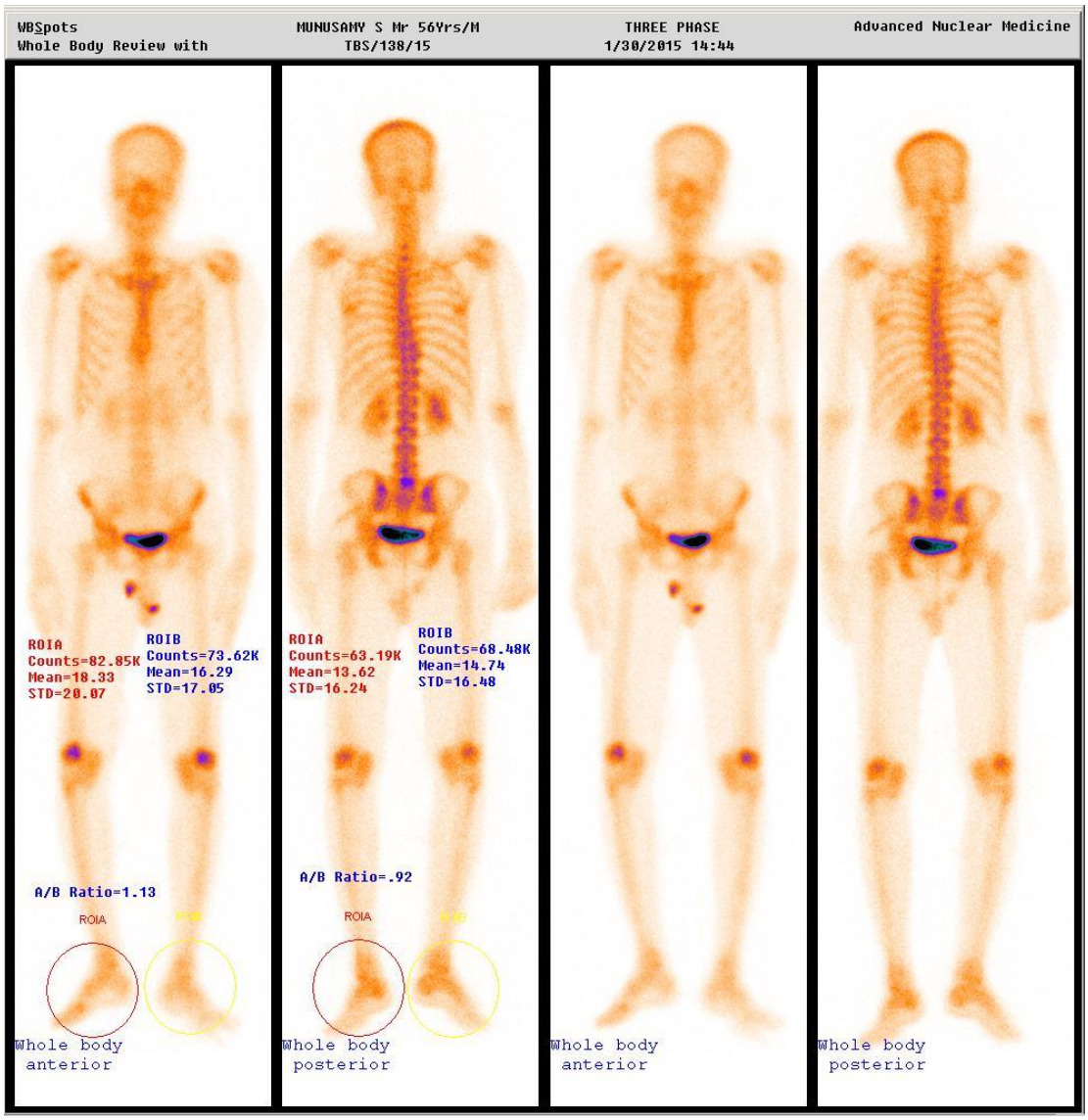
** P – Value < 0.001 Very Highly Significant

The mean values of bone uptake of Tc-99 MDP over both ankles showed a significant increase ($p<0.001$) in the study group following the Pulsed Electro Magnetic Field (PEMF) therapy.

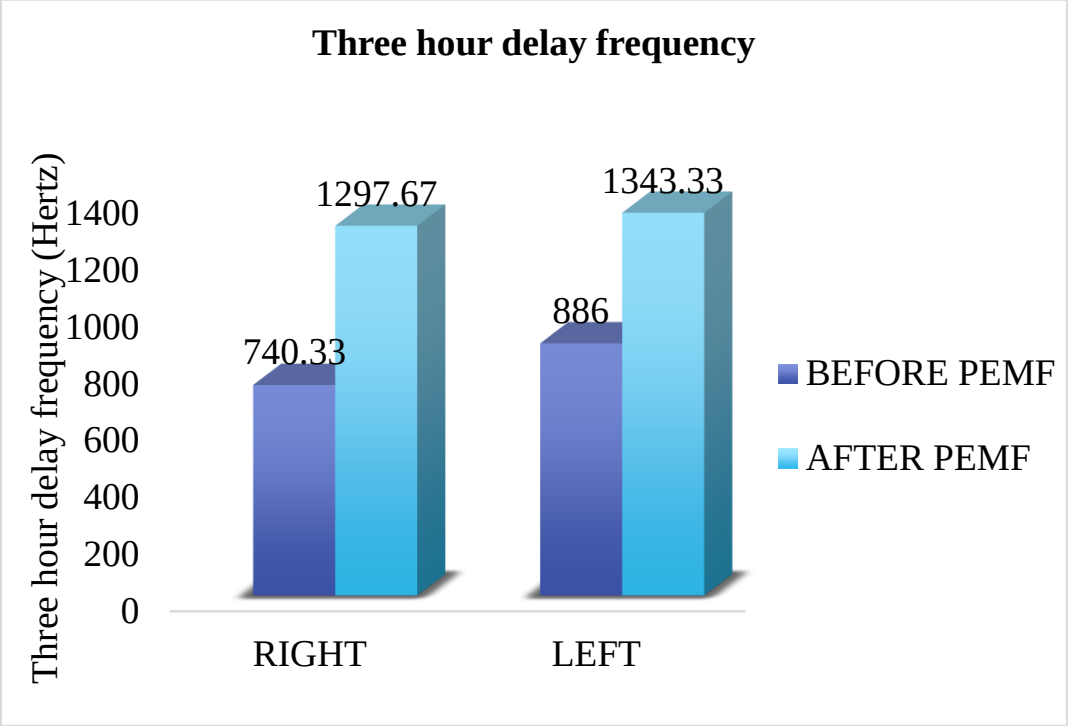
**Photograph.14.** Tc-99 MDP 3 phase bone scan (Delayed phase) report before PEMF



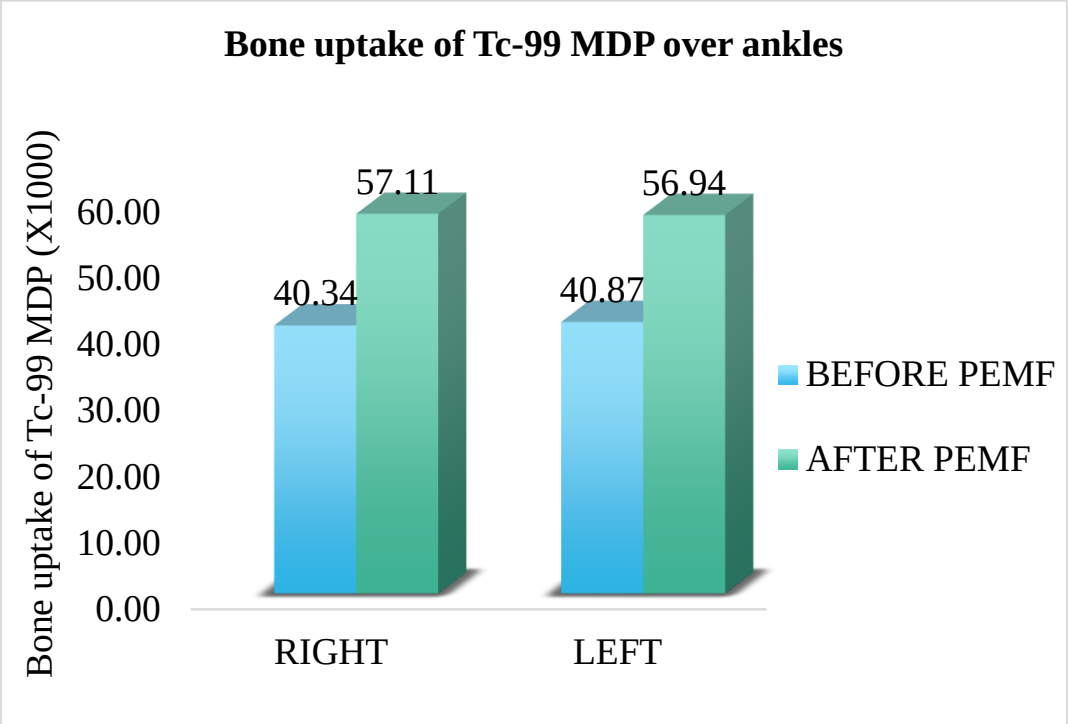
**Photograph.15.** Tc-99 MDP 3 phase bone scan (Delayed phase) report after PEMF



**Graph.11.** Comparison of three hours delay frequency of Tc-99 MDP 3 phase bone scan (Static) before and after PEMF therapy




**Graph.12.** Comparison of mean values of bone uptake of Tc-99 MDP over both ankles before and after PEMF therapy



# DISCUSSION

## DISCUSSION

The present study was done to evaluate whether low frequency Pulsed Electro Magnetic Field (PEMF) therapy in type-2 diabetes mellitus patients with sensory polyneuropathy was effective in:

a. ***Reducing neuropathic pain:*** Using Visual Analog Scale (VAS)
b. ***Influencing nerve regeneration*** using
    - Nerve conduction study in Superficial peroneal nerve
    - Vibration Perception Threshold (VPT) in foot
c. ***Improving peripheral blood flow*** using Tc-99 MDP 3 phase bone scan
d. ***Reducing oxidative stress:*** Using Erythrocyte superoxide dismutase levels

PEMF of frequency ranging between ***0.1 Hz to 20 Hz*** is capable of producing electrophysiological, neurochemical and biochemical changes in humans and animals (P.V.Sanker Narayan et al., 1985)[21]. This is also ascertained by ***Musaev et al.***[179] who reported that 10 Hz frequency PEMF was better in treating sensory symptoms of diabetic neuropathy in comparison with 100 Hz PEMF. Another study by ***Vesna Bokan Mirkovic et al.***[191] has also reported that 10 Hz frequency PEMF is more effective in treating diabetic neuropathic pain when compared to 25 Hz frequency PEMF. Hence, Pulsed Electro Magnetic Field of ***10 Hz frequency*** was used in this study.

***Reduction in Diabetic Neuropathic pain:***

Pain in diabetic neuropathy is due to abnormal repetitive firing of degenerating nociceptive afferent nerve fibres and improper functioning of sodium (Waxman SG et al., 2000[192]), calcium (Eglen RM et al., 1999[193]) and potassium (Horn S et al., 1996[194]) channels. The pain relief as evidenced by the ***significant reduction in Visual Analog Score (VAS)*** in this study may probably be due to ***partial restoration of functioning of the voltage gated ion channels***. This can be explained by hypothesis put forward by ***Panagopoulos et al.***[141] which states that externally applied electromagnetic field makes the ions to vibrate. This vibration, upon reaching a critical point, generates a ***false signal to the voltage gated channels*** present in the membranes of eukaryotic cells. This false signal, forces the voltage gated channel to either open or close affecting the cellular physiology.

Similar reduction in Visual Analog Score (VAS) in Diabetic neuropathic pain has been observed by ***Vinay Graak et al.***[176] using 600 Hz and 800 Hz for 30 minutes for 12 days. In another study by ***Wrobel et al.***[180], the diabetic neuropathic patients were exposed to low frequency magnetic fields for 20 min a day, five days a week for 3 weeks and the pain reduction did not differ significantly between the study and the control group.

The reduction in pain could also be due to ***improved peripheral circulation in foot*** as evidenced by Tc-99 MDP 3 phase bone scan in this study. Similar improvement in local circulation of the foot was also demonstrated by ***Webb et al.***[181] using 12 Hz frequency PEMF and they suggested that improved local

circulation of foot would decrease the tissue hypoxia in diabetes mellitus patients, thereby ***reducing the ischemic pain***.

***Nerve regeneration in diabetic neuropathy:***

Studies by ***Henderson et al.***[195], ***Gordon et al.***[196], ***Markov et al.***[19] have proved that PEMF can enhance nerve regeneration. ***Gordon et al.***[197], ***Ide et al.***[198], ***Johnson et al.***[199] have reported changes in ultrastructural and electrophysiological properties of regenerating peripheral nerves. These changes were demonstrated following PEMF therapy by ***Sisken et al.***[23], ***Kanje et al.***[169]**,** ***Walker et al.***[24] and ***Markov et al.***[19] thereby proving that PEMF can enhance peripheral nerve regeneration.

The ***increase in Nerve Conduction Velocity*** (NCV) and ***amplitude*** of Sensory Nerve Action Potential (SNAP) and ***decrease in latency*** of SNAP of Superficial Peroneal nerve and ***improvement in Vibration Perception Threshold (VPT)*** observed in this study could be explained by ***regenerative effect of PEMF on damaged peripheral nerves***. This result is consistent with that of the study done by ***Vinay Graak et al.***[176] except that there was no improvement in amplitude owing to lesser duration of exposure to PEMF. Also, a study by ***Weintraub et al.***[175] has shown a significant increase in Epidermal Nerve Fibre Density following PEMF therapy in diabetic neuropathy ascertaining that PEMF is capable of inducing peripheral nerve regeneration. A significant ***improvement in the Vibration Perception Threshold (VPT)*** followed by PEMF therapy observed in this study is consistent with the results of a study by ***Cieslar, G et al.***[182] which

employed sinusoidal magnetic fields for 12 minutes a day to treat diabetic neuropathy.

The probable reason for peripheral nerve regeneration produced by PEMF may be the following:

a. Recently, it has been proposed that ***CaM/nNOS/NO signalling*** has a role in ***neuronal cell survival and differentiation (***Soo-Jin Oh1 et al., 2010[149] and ***angiogenesis*** (O.M. Tepper et al., 2004[200], G. Broughton II et al., 2006[201]). Pulsed Electro Magnetic Field signals act as a '***first messenger***' that modulates Calmodulin (CaM) dependent pathways (A.A. Pilla, 2007[142]) leading to neuronal regeneration and improvement in peripheral blood flow.
b. Improvement in microcirculation due to PEMF as evidenced by ***Smith et al.***[202] as arteriolar vasodilatation in rat cremaster muscle.
c. Enhancement of '***growth factor activity and levels***' in injured nerves due to PEMF as demonstrated by ***Sisken et al.***[23], ***Longo et al.***[203]***, Macias et al.***[25]**.**

**Effect of PEMF on peripheral blood flow:**

The increase in integral percent 60-120 seconds and decrease in Time to ½ max. (Dynamic phase) of Tc-99 MDP 3 phase bone scan observed following PEMF therapy indicates that the ***blood flow has increased in the foot (region of PEMF exposure).***

The increase in frequency in blood pool phase, one hour delay and three hour delay (Static phase) and the counts of bone scan at both ankle of Tc-99 MDP 3 phase bone scan seen following PEMF therapy indicates that there is an ***improvement in peripheral blood flow to foot (region of PEMF exposure).***

This observed increase in peripheral blood flow can be attributed to angiogenesis and improvement in microcirculation produced by PEMF.

***Yen-Patton GPA et al.*** [204] have shown that PEMF stimulates the vascular endothelial cell growth in cultures. ***Smith et al.*** have demonstrated an increase of 25% in the arteriolar diameter of the rat cremaster muscle in the study group. Some microvascular studies by ***Hutchins PM et al.*** [205] and ***Yuan*** **XQ**[206] have proposed that persistent dilation of arterioles could lead to angiogenesis. A study by ***Webb et al.***[181] demonstrated an increase in peripheral blood flow and cutaneous microcirculation in foot of diabetes mellitus patients.

There is a strong evidence for the hypothesis that PEMF acts by modulating the $Ca^{2+}$ binding to Calmodulin (CaM) and affecting CaM mediated transduction pathway during a transitory increase in the intracellular calcium concentration when homeostasis is disrupted (Weissman BA et al., 2002[207]). The modulation of Ca/CaM transduction pathway by PEMF catalyzes eNOS (Nitric Oxide Synthase), which alters the release of NO from eNOS, thereby affecting the tissue repair processes like bone and tissue regeneration, neovascularisation, pain associated with it. Study by ***Roland D et al.*** [208] and ***Weber RV et al.***[209] have used an early diathermy-based RF device in an 'arterial loop model' in the rat and have found a significant increase in the angiogenesis.

PEMF was found to significantly augment tubule formation (O.M. Tepper et al., 2004[201]) in 'human umbilical vein endothelial cells' in culture secondary to production of fibroblast growth factor 2 (FGF-2). On inhibition of FGF-2, this effect was abolished, proving that ***angiogenesis by PEMF is mediated via FGF-2.*** Further, this study was extended to evaluate the effect of PEMF on 'wound repair process' in diabetic and normal mice (Callaghan MJ et al., 2008[210]) and have found PEMF induced significant increase in neovascularization, especially in diabetic mice associated with an increase in endogenous FGF-2.

**Effect of PEMF on oxidative stress:**

Erythrocyte Super Oxide Dismutase is significantly reduced in patients with Distal Symmetrical Poly Neuropathy (Gordana M. et al., 2011[211]). The probable reason for this reduced SOD activity may be glycolization of Cu, Zn-SOD (Kawamura et al., 1992[212]). A significant increase ($p<0.001$) in erythrocyte Super Oxide Dismutase levels is observed in this study. This can be due to, as suggested by ***Gordon et al.***[150], PEMF's ability to **restore "equilibrium in ROS (free radicals)/antioxidants**" by altering the alignment of the paramagnetic reactive oxygen species (ROS) free radicals like 'superoxide anion ($O_2^-$)' and 'hydroxyl anion ($OH^-$)' in the magnetic field (Zumdahl S., 1992[151]).

# CONCLUSION

## CONCLUSION

The conclusions derived from the present study are:

- Pulsed Electro Magnetic Field (PEMF) therapy has the ability to reduce neuropathic pain in painful diabetic neuropathy as evidenced by reduction of Visual Analog Score in this study.
- PEMF therapy improves the Nerve Conduction Velocity, hence plays a role in neuronal repair.
- PEMF therapy, when given for sufficient period of time (21 days) can influence nerve regeneration indicated by increase in amplitude of Sensory Nerve Action Potential in Superficial Peroneal Nerve.
- PEMF therapy focusses on pathogenesis of diabetic neuropathy, the neurovascular insufficiency. It improves in the peripheral circulation (vascularity) of foot, which in turn influences the nerve regeneration and pain alleviation.
- PEMF also has shown to reduce the oxidative stress by increasing the levels of erythrocyte Super Oxide Dismutase.
- Low frequency PEMF ***(10 Hz)*** can be used as a treatment modality for diabetic neuropathy.
- PEMF has the advantage of being non-invasive and not involving administration of any medications.
- Tc-99 MDP 3 phase bone scan be used for assessing the response to this treatment

# SUMMARY

## SUMMARY

A study was conducted to evaluate the effect of Pulsed Electro Magnetic Field therapy on type-2 diabetes mellitus patients with painful sensory polyneuropathy.

Thirty patients with painful diabetic neuropathy participated in the study. They were given PEMF therapy for 60 minutes/ day for 21 days with a break after every 6 days (protocol designed by Madras Institute of Magnetobiology). Before and after the therapy, subjects underwent a nerve conduction study in superficial peroneal nerve, vibration perception threshold was assessed, pain was assessed using visual analog score, erythrocyte Super Oxide Dismutase (SOD) levels were estimated and the vascularity of foot was assessed by Tc-99 MDP 3 phase bone scan.

The results showed a significant clinical improvement in symptoms (Pain reduction) and signs of diabetic neuropathy (Vibration Perception Threshold, Nerve Conduction study and peripheral circulation) and reduced oxidative stress following PEMF therapy. Hence, PEMF, a novel approach, can be used in treating diabetic neuropathy adjunctive to the pharmacotherapies that are currently in use.

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# BIBLIOGRAPHY

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# ANNEXURES

# INSTITUTIONAL ETHICS COMMITTEE
# MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

## CERTIFICATE OF APPROVAL

To
Dr. Charumathi Venkateshwarlu,
Post Graduate,
Institute of Physiology and Experimental Medicine,
Madras Medical College, Chennai – 600003.

Dear Dr. Charumathi Venkateshwarlu,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Evaluation of the effect of Pulsed Electro Magnetic Field Therapy in Type-2 diabetes mellitus patients with painful sensory polyneuropathy"** No.13062014

The following members of Ethics Committee were present in the meeting held on 03.06.2014 conducted at Madras Medical College, Chennai-3.

| | | |
|---|---|---|
| 1. | Dr. C. Rajendran, M.D. | -- Chairperson |
| 2. | Dr. R. Vimala, M.D., Dean, MMC, Ch-3. | -- Deputy Chair Person |
| 3. | Prof. Kalaiselvi, MD., Vice-Principal, MMC, Ch-3 | -- Member |
| 4. | Prof. Nandhini, M.D. Inst. of Pharmacology, MMC, Ch-3. | -- Member |
| 5. | Dr. G. Muralidharan, Director Incharge , Inst. of Surgery | -- Member |
| 6. | Prof. Md Ali, MD., DM., Prof & HOD of MGE, MMC, Ch-3. | -- Member |
| 7. | Prof. Ramadevi, Director i/c, Biochemistry, MMC,Ch-3. | -- Member |
| 8. | Prof. Saraswathy, MD., Director, Pathology, MMC, Ch-3. | -- Member |
| 9. | Prof. Tito, Director, i/c. Inst. of Internal Medicine, MMC | -- Member |
| 10. | Thiru. Rameshkumar, Administrative Officer | -- Lay Person |
| 11. | Thiru. S. Govindasamy, BABL, High Court, Chennai-1. | -- Lawyer |
| 12. | Tmt. Arnold Saulina, MA MSW | -- Social Scientist |

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE

Member Secretary, Ethics Committee

**INFORMED CONSENT FORM**

**Title of the study:** "Evaluation of the effect of Pulsed Electro Magnetic Field Therapy in Type-2 diabetes mellitus patients with painful sensory polyneuropathy"

**Name of the Participant:**

**Name of the Principal Investigator:** Dr. CHARUMATHI VENKATESHWARLU

**Name of the Institution:**

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

**Documentation of the informed consent**

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"**Evaluation of the effect of Pulsed Electro Magnetic Field Therapy in Type-2 diabetes mellitus patients with painful sensory polyneuropathy"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
13. I have understand that my identity will be kept confidential if my data are publicly presented.
14. I have had my questions answered to my satisfaction.
15. I have decided to be in the research study.
I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

**For adult participants:**
**Name and signature / thumb impression of the participant (or legal representative if participant incompetent**)

Name _____ Signature_____

Date_____

**Name and Signature of impartial witness (required for illiterate patients):**

Name _____Signature_____

Date_____

Address and contact number of the impartial witness:

**Name and Signature of the investigator or his representative obtaining consent:**

Name _____Signature_____

Date_____

**<u>ஆராய்ச்சி ஒப்புதல் படிவம்</u>**

***ஆராய்ச்சி தலைப்பு:*** *சர்க்கரை நோயினால் ஏற்படும் நரம்பு தளர்ச்சி மற்றும் கை, கால் எரிச்சலில் காந்த சக்தியின் விளைவுகள்*

***ஆராய்ச்சியாளர் பெயர்:*** *சாருமதி வெங்கடேஷ்வர்லு*

***ஆராய்ச்சி நடக்கும் இடம்:*** *சென்னை மருத்துவக் கல்லூரி*

**பெயர்:**

**வயது:**

***பாலினம்:*** *ஆண்/ பெண்*

***முகவரி:***

**பங்கு பெறுபவர் அடையாள எண்:**

*இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.*

*எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.*

*இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமுமின்றி சொந்த விருப்பத்தின் பேரில் சம்மதிக்கிறேன்.*

*இந்த ஆராய்ச்சியில் இருந்து நான் எந்த நேரமும் பின் வாங்கலாம் என்றும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்.*

*நான் சர்க்கரை நோயினால் ஏற்படும் நரம்பு தளர்ச்சி மற்றும் கை, கால் எரிச்சலில் காந்த சக்தியின் விளைவுகள் பற்றிய இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.*

*நான் நரம்பு கடத்துதல் பரிசோதனைக்கும் இரத்த பரிசோதனை செய்து கொள்ளவும் சம்மதிக்கிறேன்.*

*நான் என்னுடைய சுய நினைவுடன் மற்றும் முழு சம்மதத்துடன் இந்த ஆராய்ச்சிக்கு என்னை பரிசோதிக்க சம்மதிக்கிறேன்.*

*ஆராய்ச்சியாளர் கையொப்பம்* *பங்கேற்பாளர் கையொப்பம்*

*நாள்:*
*இடம்:*

**PROFORMA**

**Name:**

**Age/ Sex:**

**Address:**

**Contact number:**

**OP No.:**

**Occupation:**

Duration of diabetes mellitus: _____ Years

Duration of diabetic neuropathy: _____ Years

Treatment taken for diabetes: OHA/ Insulin

Treatment for diabetic neuropathy:

| **History of:** | Present/ absent | Duration (Years) |
|---|---|---|
| Smoking | | |
| Hypertension | | |
| Ischemic heart disease | | |
| Alcoholism | | |
| Smoking | | |
| Tuberculosis | | |
| Hansen's disease | | |

**Neuropathic symptom score of Dyck:**

**Investigations:**

| | |
|---|---|
| Blood sugar levels | mg/dl |
| Renal function tests | Urea: mg/dl, Creatinine: mg/dl |
| Complete Haemogram | Hb: g%, TC: cells/$mm^3$, DC: N L E M B<br>ESR: mm/hr, PCV: %<br>Peripheral smear: |
| Lipid profile | Total cholesterol: mg/dl, HDL: LDL: VLDL: |
| ECG | |
| Echo | |
| Fundus examination | |
| Clinical signs of peripheral vascular disease: | |

# MASTER CHART- BASELINE CHARACTERISTICS

| S.NO | Age (Years) | Sex (M/F) | Duration of diabetes mellitus (years) | Duration of diabetic neuropathy (years) | Dyck's neuropathic symptom score | Blood sugar (mg/dl) | Blood urea (mg/dl) | Serum creatinine (mg/dl) | Hb (g%) | TC (cells/mm³) | ESR (mm/hr) | PCV (%) | Peripheral smear | Total cholesterol | ECG | Echocardiography | Fundus examination |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 55 | M | 15 | 5 | 3 | 109 | 26 | 1.0 | 8.4 | 6200 | 12 | 32 | Normal | 130 | NSR | Normal study | Normal |
| 2 | 64 | F | 15 | 2 | 2 | 96 | 21 | 0.8 | 10 | 4500 | 6 | 35 | Normal | 170 | NSR | Normal study | Normal |
| 3 | 64 | M | 15 | 2 | 1 | 92 | 24 | 1.1 | 12 | 4200 | 7 | 38 | Normal | 160 | NSR | Normal study | Normal |
| 4 | 56 | F | 20 | 5 | 3 | 98 | 26 | 0.9 | 12.6 | 4400 | 7 | 39 | Normal | 149 | NSR | Normal study | Normal |
| 5 | 64 | M | 10 | 1 | 3 | 108 | 24 | 1.1 | 10 | 4500 | 8 | 36 | Normal | 160 | NSR | Normal study | Normal |
| 6 | 57 | M | 10 | 1 | 3 | 112 | 29 | 0.7 | 14 | 7000 | 12 | 39 | Normal | 180 | NSR | Normal study | Normal |
| 7 | 60 | M | 11 | 2 | 3 | 109 | 26 | 0.8 | 13 | 6400 | 8 | 40 | Normal | 132 | NSR | Normal study | Normal |
| 8 | 65 | M | 20 | 5 | 4 | 98 | 20 | 0.9 | 12 | 6800 | 6 | 39 | Normal | 150 | NSR | Normal study | Normal |
| 9 | 45 | M | 10 | 1 | 2 | 106 | 25 | 0.8 | 15 | 7100 | 6 | 45 | Normal | 140 | NSR | Normal study | Normal |
| 10 | 58 | F | 10 | 1 | 2 | 116 | 22 | 1.0 | 12 | 4300 | 8 | 39 | Normal | 164 | NSR | Normal study | Normal |
| 11 | 59 | F | 10 | 2 | 4 | 104 | 26 | 1.1 | 12 | 4800 | 8 | 38 | Normal | 166 | NSR | Normal study | Normal |
| 12 | 64 | F | 10 | 1 | 1 | 94 | 26 | 1.2 | 10 | 5300 | 9 | 34 | Normal | 153 | NSR | Normal study | Normal |
| 13 | 60 | F | 22 | 1 | 3 | 131 | 19 | 1.0 | 11.8 | 8000 | 5 | 38 | Normal | 155 | NSR | Normal study | Normal |
| 14 | 56 | M | 10 | 1 | 3 | 98 | 22 | 0.9 | 13 | 5600 | 8 | 42 | Normal | 160 | NSR | Normal study | Normal |
| 15 | 63 | M | 10 | 1 | 3 | 110 | 24 | 1.1 | 12 | 4400 | 8 | 36 | Normal | 144 | NSR | Normal study | Normal |
| 16 | 55 | M | 10 | 1 | 2 | 96 | 22 | 0.7 | 11 | 6300 | 6 | 37 | Normal | 142 | NSR | Normal study | Normal |
| 17 | 58 | M | 15 | 1 | 2 | 135 | 25 | 0.8 | 11.5 | 5700 | 10 | 36 | Normal | 150 | NSR | Normal study | Normal |
| 18 | 52 | F | 12 | 1 | 3 | 142 | 20 | 1.0 | 11 | 6100 | 7 | 34 | Normal | 136 | NSR | Normal study | Normal |
| 19 | 62 | M | 23 | 3 | 3 | 127 | 21 | 0.7 | 12 | 6250 | 8 | 38 | Normal | 156 | NSR | Normal study | Normal |
| 20 | 60 | M | 20 | 1 | 4 | 152 | 20 | 0.7 | 11.5 | 7200 | 8 | 38 | Normal | 142 | NSR | Normal study | Normal |
| 21 | 47 | F | 15 | 1 | 3 | 124 | 20 | 0.6 | 12.5 | 8100 | 11 | 39 | Normal | 131 | NSR | Normal study | Normal |
| 22 | 60 | F | 15 | 2 | 2 | 138 | 23 | 0.7 | 11 | 7250 | 9 | 38 | Normal | 172 | NSR | Normal study | Normal |
| 23 | 50 | F | 15 | 3 | 3 | 145 | 23 | 0.9 | 12 | 7300 | 10 | 40 | Normal | 144 | NSR | Normal study | Normal |
| 24 | 51 | M | 16 | 5 | 3 | 127 | 20 | 0.6 | 11.5 | 6100 | 8 | 37 | Normal | 148 | NSR | Normal study | Normal |
| 25 | 58 | F | 10 | 1 | 3 | 115 | 23 | 0.7 | 11.8 | 5250 | 10 | 42 | Normal | 153 | NSR | Normal study | Normal |
| 26 | 48 | M | 10 | 6 | 3 | 126 | 22 | 0.8 | 11 | 7500 | 12 | 36 | Normal | 146 | NSR | Normal study | Normal |
| 27 | 52 | F | 20 | 5 | 3 | 105 | 23 | 0.6 | 12 | 7250 | 10 | 37 | Normal | 192 | NSR | Normal study | Normal |
| 28 | 62 | F | 10 | 1 | 4 | 92 | 24 | 0.7 | 11.5 | 4500 | 12 | 38 | Normal | 209 | NSR | Normal study | Normal |
| 29 | 48 | F | 12 | 3 | 3 | 124 | 24 | 0.8 | 12 | 5700 | 8 | 37 | Normal | 206 | NSR | Normal study | Normal |
| 30 | 60 | F | 15 | 2 | 4 | 114 | 22 | 0.7 | 11.5 | 4900 | 7 | 36 | Normal | 148 | NSR | Normal study | Normal |

# MASTER CHART- BEFORE PEMF

| S.NO | Age (Years) | Sex (M/F) | Nerve conduction study in superficial peroneal nerve | | | | | | Tc 99 MDP scan | | | | | | | | | | | | SOD levels (U/g Hb) | Vibration Perception Threshold (Volts) | | VAS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Amplitude (Microvolts) | | Distal latency (Milliseconds) | | Nerve conduction velocity (m/sec) | | Integral 60-120 seconds (1000c/sec) | | Time to ½ max. (Seconds) | | Blood pool frequency (Hz) | | 1 hour delay frequency (Hz) | | 3 hours delay frequency (Hz) | | Bone uptake counts (X1000) | | | | | |
| | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | | Right | Left | |
| 1 | 55 | M | 11.4 | 11.8 | 3.4 | 3.62 | 35.29 | 33.15 | 21.6 | 20.6 | 22.1 | 22.1 | 700 | 780 | 800 | 900 | 800 | 780 | 49.32 | 60.71 | 343 | 22.8 | 19.7 | 8 |
| 2 | 64 | F | 12.75 | 12.95 | 3.54 | 3.73 | 33.90 | 32.17 | 7.8 | 6.8 | 31.6 | 32.5 | 1300 | 1100 | 1400 | 1700 | 1400 | 1900 | 41.09 | 30.01 | 331 | 15 | 15.5 | 10 |
| 3 | 64 | M | 12.41 | 13.72 | 3.98 | 3.7 | 30.15 | 32.43 | 4.9 | 6.2 | 59.6 | 45.4 | 1400 | 1600 | 880 | 900 | 1500 | 1600 | 28.19 | 31.17 | 320 | 27.5 | 25.8 | 8 |
| 4 | 56 | F | 12.47 | 12.56 | 3.75 | 3.87 | 32.00 | 31.01 | 2.9 | 3.2 | 49.3 | 47.6 | 110 | 140 | 440 | 460 | 460 | 480 | 25.32 | 28.12 | 351 | 19.3 | 20.6 | 8 |
| 5 | 64 | M | 10.15 | 10.98 | 5.05 | 4.42 | 23.76 | 27.15 | 4.6 | 4.2 | 23.2 | 23.7 | 480 | 380 | 360 | 360 | 340 | 400 | 27.63 | 25.56 | 333 | 43 | 43 | 10 |
| 6 | 57 | M | 10.45 | 10.64 | 4.98 | 4.87 | 24.10 | 24.64 | 3.5 | 4.2 | 50.8 | 48.6 | 460 | 480 | 480 | 500 | 440 | 460 | 29.65 | 32.34 | 312 | 45 | 45 | 10 |
| 7 | 60 | M | 10.33 | 10.62 | 5.1 | 5.32 | 23.53 | 22.56 | 2.1 | 2.4 | 33.6 | 46.6 | 850 | 850 | 350 | 450 | 350 | 400 | 22.69 | 25.93 | 339 | 43 | 43 | 10 |
| 8 | 65 | M | 10.51 | 10.24 | 5.02 | 5.21 | 23.90 | 23.03 | 2.3 | 1.8 | 32.7 | 48.3 | 620 | 450 | 600 | 550 | 420 | 300 | 25.84 | 25.79 | 316 | 43 | 43 | 10 |
| 9 | 45 | M | 10.37 | 10.21 | 5.14 | 4.88 | 23.35 | 24.59 | 5.8 | 2.6 | 21.7 | 42.9 | 800 | 750 | 800 | 1100 | 1500 | 1300 | 37.55 | 42.93 | 341 | 43 | 43 | 10 |
| 10 | 58 | F | 10.19 | 10.93 | 4.53 | 4.29 | 26.49 | 27.97 | 3.2 | 2.8 | 29.6 | 31.7 | 400 | 260 | 260 | 260 | 200 | 300 | 19.89 | 21.94 | 330 | 43 | 43 | 9 |
| 11 | 59 | F | 10.73 | 11.02 | 4.68 | 4.12 | 25.64 | 29.13 | 17.2 | 17.6 | 28.3 | 37.9 | 480 | 520 | 800 | 900 | 600 | 700 | 55.39 | 48.72 | 345 | 43 | 43 | 10 |
| 12 | 64 | F | 11.4 | 11.8 | 3.4 | 3.62 | 35.29 | 33.15 | 9.2 | 9.2 | 19.5 | 14 | 1000 | 1100 | 500 | 600 | 1000 | 1300 | 34.3 | 17.81 | 321 | 35 | 35 | 10 |
| 13 | 60 | F | 10.02 | 9.63 | 4.59 | 4.73 | 26.14 | 25.37 | 6.1 | 2.3 | 20.8 | 45.9 | 920 | 1200 | 850 | 1000 | 1400 | 1800 | 38.66 | 43.1 | 333 | 43 | 43 | 9 |
| 14 | 56 | M | 10.23 | 10.77 | 4.34 | 4.01 | 27.65 | 29.93 | 4.1 | 4 | 45.2 | 47.4 | 380 | 420 | 500 | 500 | 380 | 500 | 26.59 | 24.52 | 318 | 44 | 39 | 10 |
| 15 | 63 | M | 12.59 | 13.60 | 3.74 | 3.03 | 32.09 | 39.60 | 3.4 | 2.8 | 48.7 | 46.5 | 200 | 200 | 180 | 420 | 180 | 300 | 18.26 | 21.78 | 328 | 26.5 | 17.5 | 9 |
| 16 | 55 | M | 10.24 | 12.63 | 4.49 | 3.47 | 26.73 | 34.58 | 4.9 | 4.8 | 21.4 | 19.5 | 480 | 500 | 100 | 118 | 900 | 1100 | 19.98 | 21.07 | 331 | 45 | 20 | 10 |
| 17 | 58 | M | 12.72 | 11.95 | 3.28 | 3.79 | 36.59 | 31.66 | 19.98 | 21.07 | 72.8 | 68 | 510 | 580 | 700 | 850 | 580 | 700 | 32.8 | 22.36 | 345 | 25 | 26 | 10 |
| 18 | 52 | F | 12.96 | 12.53 | 3.15 | 3.56 | 38.10 | 33.71 | 12.1 | 11 | 20.6 | 17.6 | 420 | 340 | 110 | 158 | 360 | 480 | 39.86 | 45.27 | 342 | 14.2 | 19.2 | 10 |
| 19 | 62 | M | 10.19 | 10.27 | 4.83 | 5.12 | 24.84 | 23.44 | 18.4 | 19.3 | 27.6 | 32.8 | 500 | 540 | 800 | 1000 | 620 | 750 | 25.95 | 25.1 | 318 | 44 | 44 | 9 |
| 20 | 60 | M | 10.79 | 10.98 | 4.72 | 4.95 | 25.42 | 24.24 | 11.4 | 12.9 | 37.4 | 45.7 | 300 | 1750 | 600 | 1500 | 700 | 1400 | 40.45 | 39.4 | 326 | 42 | 42 | 9 |
| 21 | 47 | F | 11.44 | 11.86 | 3.99 | 3.67 | 30.08 | 32.70 | 6.7 | 5.9 | 22.5 | 24.1 | 1200 | 1300 | 520 | 500 | 410 | 450 | 79.6 | 93.7 | 323 | 28.7 | 21.8 | 9 |
| 22 | 60 | F | 11.02 | 11.52 | 4.18 | 3.78 | 28.71 | 31.75 | 5.3 | 5.1 | 46.7 | 48.9 | 400 | 450 | 550 | 550 | 400 | 550 | 28.13 | 27.44 | 339 | 40.3 | 34.3 | 10 |
| 23 | 50 | F | 10.03 | 10.15 | 4.96 | 4.81 | 24.19 | 24.95 | 2.4 | 1.3 | 27.1 | 57.1 | 600 | 780 | 700 | 650 | 400 | 320 | 63.21 | 56.13 | 324 | 45 | 45 | 10 |
| 24 | 51 | M | 11.08 | 10.91 | 3.83 | 4.01 | 31.33 | 29.93 | 7 | 6.6 | 35.9 | 36.6 | 1300 | 1400 | 700 | 680 | 500 | 520 | 55.23 | 59.77 | 327 | 28.2 | 37.6 | 9 |
| 25 | 58 | F | 11.39 | 11.07 | 3.66 | 3.94 | 32.79 | 30.46 | 6.7 | 8.4 | 48 | 46 | 720 | 840 | 720 | 720 | 900 | 1200 | 42.54 | 48.72 | 340 | 25.6 | 29.8 | 10 |
| 26 | 48 | M | 10.32 | 10.25 | 4.93 | 5.03 | 24.34 | 23.86 | 8.1 | 8.7 | 20.8 | 18.6 | 1300 | 1500 | 1250 | 900 | 1600 | 2000 | 61.34 | 51.43 | 314 | 43 | 43 | 10 |
| 27 | 52 | F | 11.05 | 10.96 | 4.51 | 4.63 | 26.61 | 25.92 | 8.2 | 7.1 | 32.7 | 30.9 | 1200 | 1100 | 1200 | 1500 | 1300 | 1400 | 54.21 | 58.91 | 338 | 38.3 | 41.7 | 10 |
| 28 | 62 | F | 11.93 | 11.26 | 3.85 | 4.06 | 31.17 | 29.56 | 4.2 | 3.1 | 26.5 | 37.5 | 420 | 400 | 480 | 600 | 250 | 390 | 59.17 | 62.62 | 328 | 23.3 | 26.7 | 9 |
| 29 | 48 | F | 11.76 | 11.15 | 3.92 | 4.15 | 30.61 | 28.92 | 7.2 | 8.1 | 39 | 37.9 | 700 | 820 | 700 | 700 | 920 | 1000 | 53.96 | 55.84 | 316 | 25.7 | 29.8 | 9 |
| 30 | 60 | F | 10.57 | 10.69 | 4.98 | 5.17 | 24.10 | 23.21 | 9.3 | 10.2 | 18.4 | 19.3 | 1100 | 1300 | 1200 | 1000 | 1400 | 1800 | 73.44 | 77.85 | 320 | 43 | 43 | 10 |

# MASTER CHART-AFTER PEMF

| S.NO | Age (Years) | Sex (M/F) | Nerve conduction study in superficial peroneal nerve | | | | | | Tc 99 MDP scan | | | | | | | | | | | | SOD levels (U/g Hb) | Vibration Perception Threshold (Volts) | | VAS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Amplitude (Microvolts) | | Distal latency (Milliseconds) | | Nerve conduction velocity (m/sec) | | Integral 60-120 seconds (1000c/sec) | | Time to ½ max. (Seconds) | | Blood pool frequency (Hz) | | 1 hour delay frequency (Hz) | | 3 hours delay frequency (Hz) | | Bone uptake counts (X1000) | | | | | |
| | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | | Right | Left | |
| 1 | 55 | M | 17.57 | 18.3 | 1.95 | 2.1 | 61.54 | 57.14 | 23.2 | 23.5 | 13.2 | 13.6 | 1750 | 2300 | 1250 | 2100 | 2100 | 2400 | 177 | 157.8 | 365 | 8.5 | 8 | 0 |
| 2 | 64 | F | 18.14 | 18.98 | 3.03 | 2.05 | 39.60 | 58.54 | 12.3 | 10.5 | 5.2 | 6.1 | 2100 | 1800 | 2700 | 2250 | 2800 | 2800 | 57.87 | 39.68 | 372 | 8 | 9.3 | 2 |
| 3 | 64 | M | 17.97 | 19.01 | 2.3 | 1.83 | 52.17 | 65.57 | 7.2 | 12.1 | 25.5 | 27.7 | 1800 | 1900 | 900 | 1100 | 2200 | 2000 | 49.32 | 60.71 | 388 | 12.3 | 12.6 | 0 |
| 4 | 56 | F | 17.78 | 18.05 | 2.15 | 1.98 | 55.81 | 60.61 | 10.6 | 12.6 | 23.6 | 21.8 | 400 | 450 | 600 | 640 | 620 | 600 | 30.89 | 34.76 | 379 | 11.6 | 8.3 | 2 |
| 5 | 64 | M | 17.62 | 17.93 | 3.17 | 2.19 | 37.85 | 54.79 | 5.6 | 4.9 | 10.5 | 15.3 | 750 | 700 | 800 | 750 | 650 | 700 | 29.41 | 34.79 | 382 | 25 | 10 | 0 |
| 6 | 57 | M | 16.53 | 16.68 | 3.05 | 2.79 | 39.34 | 43.01 | 8.8 | 9.6 | 24.9 | 21.3 | 700 | 780 | 860 | 900 | 760 | 800 | 42.98 | 45.88 | 370 | 35.8 | 27.5 | 0 |
| 7 | 60 | M | 16.98 | 16.77 | 3.24 | 3.27 | 37.04 | 36.70 | 4.3 | 3.1 | 22 | 23.2 | 980 | 920 | 1250 | 1100 | 880 | 920 | 37.38 | 39.56 | 366 | 34.8 | 35 | 0 |
| 8 | 65 | M | 16.83 | 16.27 | 3.46 | 3.18 | 34.68 | 37.74 | 9.6 | 7.8 | 15 | 14.7 | 1200 | 1000 | 1400 | 1200 | 780 | 700 | 27.28 | 23.82 | 388 | 26.6 | 26.6 | 2 |
| 9 | 45 | M | 16.72 | 16.24 | 3.59 | 2.82 | 33.43 | 42.55 | 11.2 | 6.4 | 8.5 | 10.3 | 1250 | 1400 | 1000 | 1500 | 2000 | 1800 | 42.02 | 46.87 | 369 | 29 | 19.1 | 0 |
| 10 | 58 | F | 17.05 | 17.67 | 2.95 | 2.56 | 40.68 | 46.88 | 9.7 | 9 | 20.3 | 20.1 | 650 | 450 | 550 | 500 | 450 | 480 | 24.32 | 27.83 | 385 | 26.6 | 24.2 | 0 |
| 11 | 59 | F | 16.19 | 17.82 | 3.19 | 2.39 | 37.62 | 50.21 | 22.1 | 20.8 | 21.7 | 21.2 | 1400 | 1400 | 1100 | 1100 | 1600 | 1300 | 66.94 | 57.4 | 371 | 17.3 | 20 | 0 |
| 12 | 64 | F | 17.24 | 17.93 | 2.79 | 2.15 | 43.01 | 55.81 | 9.4 | 9.3 | 10.4 | 7.4 | 1400 | 1600 | 1200 | 1250 | 1500 | 1500 | 36.93 | 20.31 | 380 | 10.2 | 10 | 0 |
| 13 | 60 | F | 16.86 | 17.14 | 2.99 | 2.74 | 40.13 | 43.80 | 10.9 | 5.9 | 4.2 | 55.4 | 1400 | 1400 | 900 | 1400 | 1800 | 1800 | 40.89 | 45.24 | 386 | 15 | 15 | 0 |
| 14 | 56 | M | 17.23 | 17.85 | 2.76 | 2.19 | 43.48 | 54.79 | 13.2 | 11.4 | 23.4 | 21.5 | 700 | 950 | 700 | 700 | 800 | 850 | 82.85 | 73.62 | 363 | 5 | 5 | 0 |
| 15 | 63 | M | 17.96 | 18.04 | 2.06 | 1.93 | 58.25 | 62.18 | 10.2 | 11.8 | 21.9 | 20.4 | 400 | 380 | 480 | 600 | 360 | 480 | 25.31 | 28.72 | 369 | 10 | 10.8 | 0 |
| 16 | 55 | M | 17.15 | 17.93 | 2.89 | 2.17 | 41.52 | 55.30 | 10.1 | 8.1 | 18.3 | 15.3 | 500 | 520 | 900 | 1250 | 1000 | 1250 | 29.62 | 28.91 | 384 | 18.3 | 15 | 2 |
| 17 | 58 | M | 18.92 | 18.07 | 1.87 | 2.29 | 64.17 | 52.40 | 29.62 | 28.91 | 21.4 | 25 | 620 | 1000 | 1100 | 1200 | 600 | 780 | 32.78 | 24.94 | 379 | 15 | 15 | 0 |
| 18 | 52 | F | 18.47 | 17.94 | 1.79 | 2.23 | 67.04 | 53.81 | 20.4 | 20.3 | 20.5 | 16.5 | 700 | 780 | 380 | 380 | 580 | 580 | 71.69 | 80.39 | 366 | 10 | 10 | 0 |
| 19 | 62 | M | 16.39 | 16.07 | 3.16 | 3.95 | 37.97 | 30.38 | 25.9 | 28.6 | 20.9 | 21.4 | 1200 | 1250 | 1250 | 1300 | 1500 | 1600 | 33.97 | 36.25 | 376 | 20.8 | 20.8 | 2 |
| 20 | 60 | M | 16.14 | 17.12 | 3.02 | 2.82 | 39.74 | 42.55 | 14.8 | 18.4 | 26.1 | 34.8 | 680 | 2600 | 900 | 2000 | 1000 | 1800 | 51.8 | 60.36 | 362 | 10 | 10 | 2 |
| 21 | 47 | F | 17.29 | 18.03 | 2.83 | 2.06 | 42.40 | 58.25 | 10 | 9.2 | 7.3 | 15.6 | 1500 | 1600 | 1400 | 1400 | 1200 | 1200 | 84.97 | 97.62 | 387 | 10.8 | 10 | 1 |
| 22 | 60 | F | 17.37 | 18.36 | 2.96 | 2.18 | 40.54 | 55.05 | 14.6 | 12.8 | 22.8 | 23.6 | 750 | 800 | 850 | 900 | 900 | 1000 | 48.67 | 47.4 | 381 | 15.8 | 13.3 | 0 |
| 23 | 50 | F | 16.21 | 17.65 | 3.27 | 2.86 | 36.70 | 41.96 | 9.8 | 8 | 12 | 13.6 | 2000 | 2500 | 900 | 1000 | 800 | 680 | 77.92 | 68.09 | 373 | 13.3 | 15.8 | 0 |
| 24 | 51 | M | 17.81 | 17.69 | 2.14 | 2.32 | 56.07 | 51.72 | 14 | 13.4 | 20.5 | 24.3 | 1600 | 1800 | 1000 | 1200 | 900 | 880 | 64.98 | 68.65 | 374 | 10.8 | 13.3 | 2 |
| 25 | 58 | F | 18.09 | 17.83 | 2.02 | 2.29 | 59.41 | 52.40 | 8.5 | 10.1 | 17 | 17.8 | 1400 | 1400 | 1800 | 1800 | 2000 | 2000 | 51.95 | 55.87 | 377 | 10 | 10 | 0 |
| 26 | 48 | M | 15.92 | 15.17 | 3.41 | 3.92 | 35.19 | 30.61 | 32.7 | 34.2 | 6 | 7 | 1800 | 1800 | 1800 | 2500 | 1800 | 2100 | 76.87 | 65.47 | 376 | 19.2 | 22.5 | 2 |
| 27 | 52 | F | 16.24 | 17.13 | 3.11 | 2.62 | 38.59 | 45.80 | 13.7 | 11.9 | 8.2 | 7.3 | 1800 | 1600 | 2000 | 2200 | 2200 | 2000 | 74.31 | 80.07 | 390 | 16.3 | 25.8 | 0 |
| 28 | 62 | F | 16.96 | 17.58 | 2.74 | 2.08 | 43.80 | 57.69 | 6.7 | 4.5 | 18.2 | 22.6 | 1800 | 2200 | 2400 | 2600 | 1400 | 1500 | 93.45 | 98.17 | 372 | 14.2 | 17.5 | 0 |
| 29 | 48 | F | 17.37 | 17.98 | 2.86 | 2.15 | 41.96 | 55.81 | 9.5 | 11.5 | 16 | 15.2 | 1200 | 1400 | 1500 | 1500 | 1750 | 1800 | 62.06 | 68.93 | 386 | 17.5 | 19.2 | 0 |
| 30 | 60 | F | 16.43 | 16.02 | 3.17 | 3.92 | 37.85 | 30.61 | 31.6 | 35.8 | 8.2 | 7.3 | 1400 | 1500 | 1800 | 1800 | 2000 | 2000 | 86.73 | 90.05 | 380 | 26.7 | 25.8 | 2 |